

OPERATIVE DENTISTRY

Nov/Dec 2021

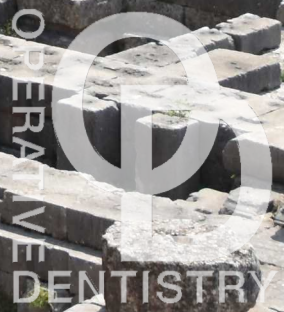
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Aim and Scope

Operative Dentistry publishes articles that advance the practice of operative dentistry. The scope of the journal includes conservation and restoration of teeth; the scientific foundation of operative dental therapy; dental materials; dental education; and the social, political, and economic aspects of dental practice. Review papers, book reviews, letters and classified ads for faculty positions are also published.

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Operative Dentistry, Inc. would like to thank our conscientious team of Reviewers for their hard work, tenacity, and dedication in the furthering of operative dentistry around the world. These individuals dedicate innumerable hours in reading, re-reading, and critiquing manuscripts. Submitted articles, accepted for publication or not, all benefit from these reviewers who help authors present their hard work, as well as verifying that the work we publish is scientifically accurate, clinically relevant, and professionally uplifting. We want to publicly recognize them, although with the knowledge that it would never be possible to thank them enough for their contributions. Thank you.

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Adhesive Management of Anterior Tooth Wear in Combination with the Dahl Concept—A 27-Month Observational Case Series

E Lempel • KG Németh • BV Lovász • J Szalma

Clinical Relevance

Localized anterior tooth wear can be restored with adhesively bonded labial ceramic and palatal direct resin composite veneers at an increased vertical dimension of occlusion. This additive technique provides maximal tooth preservation for a worn dentition.

SUMMARY

Localized anterior maxillary tooth wear caused by erosion and attrition with loss of interocclusal space is difficult to manage.

This observational case-series study reports six cases with worn anterior dentition treated with labial ceramic and palatal direct resin composite veneers at an increased vertical dimension of occlusion without restoration of unaffected posterior teeth.

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Thirty-six palatal direct veneers were made in six patients from a nanohybrid resin composite with the help of a wax-up-based template at an increased vertical dimension. After the complete re-establishment of posterior occlusion, 40 labial lithium-disilicate ceramic veneers were fabricated with a mock-up-guided method. The sandwich veneers were evaluated according to the United States Public Health Service (USPHS) criteria after a mean service time of 22.7 months. Re-establishment of posterior contacts as well as subjective patient satisfaction and function were evaluated. The overall success of the labial ceramic veneers was excellent. The quality of the palatal resin composite restorations was found to be good with predominantly “Alpha” scores. The marginal quality (11.1% and 33.3% of integrity and discoloration, respectively) and surface roughness (16.7%) showed small deteriorations indicated by “Beta” scores. The resin composite showed, in general, signs of wear facets which resulted in “Beta” scores in 44.4% of the cases. Posterior contacts re-established firmly within 4 weeks in all cases. Patient satisfaction with esthetics and function was high. The short-

term outcome of this non-invasive treatment option is favorable and promising.

INTRODUCTION

The general term for non-carious tooth loss is tooth wear and describes loss of dental hard tissue that can be generated by different etiological factors such as chemical and mechanical processes.¹ According to Loomans and others, tooth wear is pathological if it is atypical for the age of the patient or causes pain or discomfort, functional problems, or deterioration of esthetic appearance; if it progresses, it may give rise to undesirable complications of increasing complexity.² Tooth wear can be identified as erosion, attrition, abrasion, and abfraction.³ Clinical observations have demonstrated that wear mechanisms do not act alone but instead cause loss of tooth surfaces by interacting with each other.⁴ An increased incidence of erosive wear is being reported in young populations thanks to their dietary habits, the increased consumption of soft drinks and energy drinks, and the stressful effects of an accelerated lifestyle.⁵ Irrespective of etiological factors, tooth wear can influence oral health and personal comfort and may result in loss of vertical dimension of occlusion (VDO) and in tooth sensitivity and pulpal involvement. However, especially for young adult patients, esthetic complaints are paramount.^{6,7} Also, depending on the progression of tooth wear, incisal edges may show signs of wear and shortening due to loss of enamel support and subsequent chipping. The rate of anterior erosive tooth wear can be severe, even at an early age, if bruxism or other parafunction occurs alone or concurrently with erosion.⁸

The management of tooth wear may involve complex preventive and restorative care, possibly with full rehabilitation at an increased vertical dimension of occlusion.⁹⁻¹¹ Currently, adhesive dentistry is undergoing considerable progress, enabling advanced restoration techniques. In the restorative management of tooth wear, there has been a tendency towards minimum-intervention approaches.² Following the minimally invasive treatment concept, partial-covering, all-ceramic restorations and direct-resin-based composite (RBC) have recently become recommended as preferred therapy.¹²⁻¹⁶ Mesko and others included several papers in their systematic review of the materials and techniques used to restore teeth with severe tooth wear.¹⁷ They concluded that the absence of randomized clinical trials on the subject appears to be the main limitation of the existing evidence. However, in line with Ahmed and others, Mesko and others also

found RBC to be a feasible option to restore teeth with severe wear and demonstrated good performance of this material over short- and medium-term follow-up.^{17,18} Unlike the traditional subtractive techniques, RBC, with the help of reliable adhesion to the tooth, provides additive treatment solutions while preserving the remaining tooth structure.² Vailati and Belser presented a three-step technique to reconstruct eroded anterior teeth with an additive adhesive approach, providing maximum preservation of tooth structure with a predictable esthetic and functional outcome.¹⁹⁻²¹ The applied sandwich veneers provide palatal guidance and buccal esthetics with the protection of the remaining tooth structure as well as adhesive increase of VDO in the molar region.¹⁹⁻²¹

An ultraconservative and simplified treatment is proposed, using the combination of centric relation and the Dahl principle in the solution of localized anterior tooth wear and erosion.²² In localized anterior tooth wear, compensatory eruption may take place, maintaining the VDO despite the loss of anterior clinical crown height.²³ The resultant loss of interocclusal space presents a challenge for restoration, especially when the posterior teeth are unaffected. The Dahl approach consists of creating interocclusal space through axial tooth movement, with an appliance or restorations placed in supra-occlusion, and the subsequent re-establishment of full arch occlusal contacts over a period of time.^{24,25} The original Dahl appliance was based on a metal cobalt-chromium appliance cemented on the palatal surfaces of upper anterior teeth; however, more recently RBC restorations have been used to create the space with good short- to medium-term survival.²⁵⁻²⁸ Although the clinical performance seems satisfactory, the limited mechanical and physical properties of RBC restorations demand maintenance.²⁸ To overcome the esthetic issue that arises from the deterioration of RBC, ceramic veneers can be used on the labial surfaces of anterior teeth, following the perceived needs and concerns of the patient. Meanwhile, the RBC on the palatal surface can withstand the increased functional stress at an increased VDO. At the level of case reports, this sandwich approach, in combination with the Dahl principle, was introduced by Magne and others, but there is a lack of data about the short-, medium- and long-term performance of this biomechanically favorable treatment option.²²

The aim of this prospective, observational case-series study was to assess the short-term clinical performance and patient satisfaction associated with

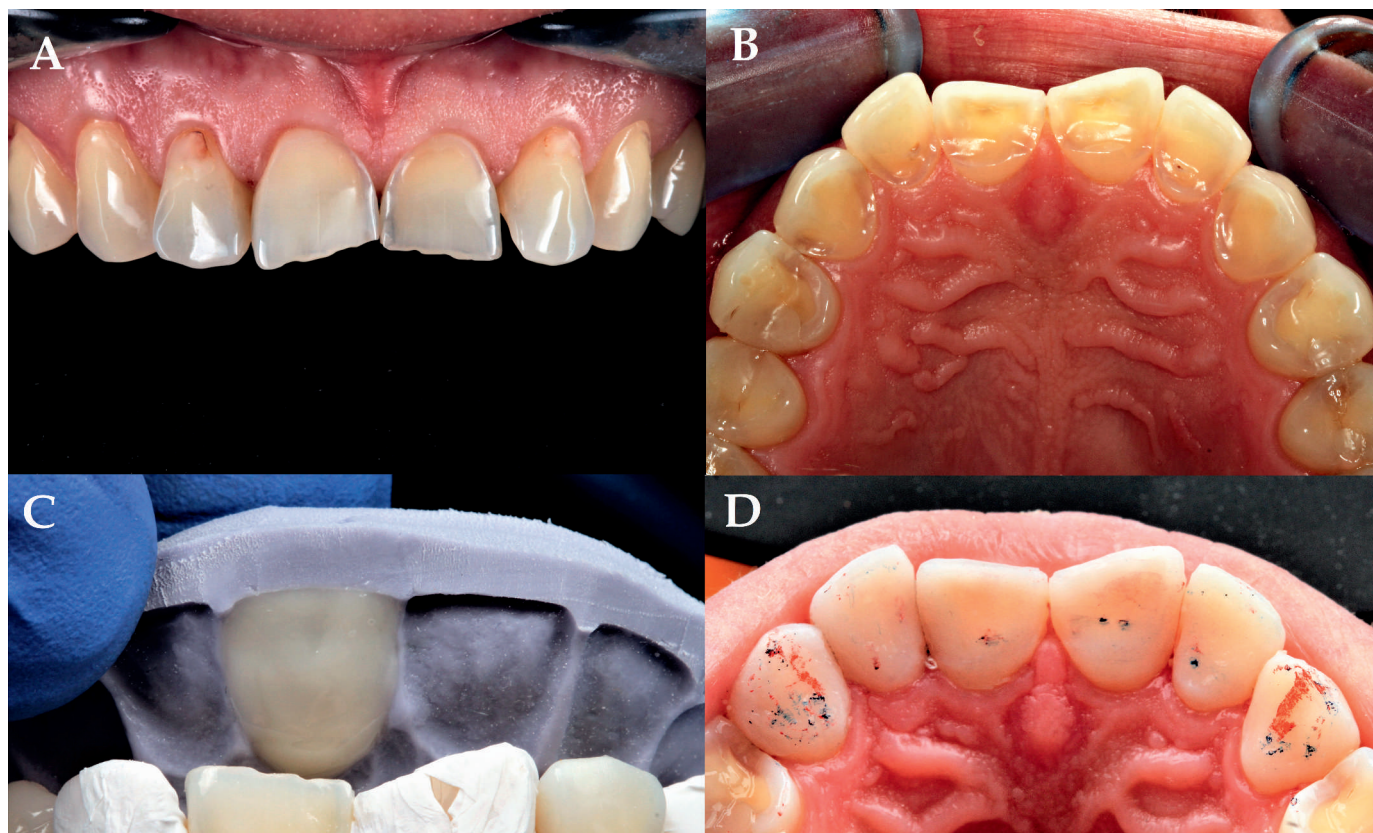


Figure 1. Initial situation of localized anterior tooth wear from labial (A) and palatal (B) aspects. The process of the palatal direct resin composite veneer fabrication by silicone matrix technique (C) providing static and dynamic contacts on the anterior teeth (D).

a sandwich approach to the treatment of localized anterior tooth wear, consisting of palatal resin composite direct build-ups and vestibular lithium-disilicate veneers in combination with the Dahl approach.

METHODS & MATERIALS

Patient selection

For this prospective, observational case-series study a total of six patients (two males and four females) aged 22 to 34 years were selected according to predetermined inclusion criteria. All participants were required to be adults able to read and sign the informed consent document and to be physically and psychologically able to tolerate the procedure. In addition, patients selected for the study needed to have good oral hygiene, full dentition, and normal occlusion and to be without periodontal disease, as verified by clinical and radiographic records. Moreover, patients were required to remain in continuous clinical follow-up without attending other dentists. The minimal length of the follow-up period was 20 months. Reasons for placement of direct palatal RBC

veneers and labial ceramic veneers were either localized anterior attrition or combination of erosion and attrition with relatively unaffected posterior teeth (Figure 1A, 1B). Tooth wear was determined to be clinically significant when dentin was exposed and when there was a reduction of crown height. Margins placed on enamel with a vital pulp were also required for the placement of direct and indirect veneers.

The patients were seeking advice for esthetic reasons, to improve their deteriorated and socially disadvantageous smiles. Before the written informed consent was signed, patients were given a full explanation of the proposed treatment. They were treated at the Operative Dentistry Department of the University of Pécs by one operator. The cause of the localized anterior tooth wear was identified as closely as possible, based on a careful history and examination. The etiology was primarily attrition (bruxism-related occlusal stress) in half of the cases and combined erosion/attrition (confirmed and treated gastroesophageal reflux disease in two cases and a high rate of soft drink/energy drink consumption in

one case with concurrent bruxism-related wear) in the other half (Table 1).

Bruxism-related occlusal stress was identified by self-report and clinical examination. The etiology of bruxism related to anxiety, insomnia, and lifestyle-related stress. Elimination of the etiological factors was being started or was ongoing.

History taking was performed by a questionnaire survey developed by Pintado and others that focused on nocturnal or awake tooth grinding, jaw fatigue, or temporal headache on awakening.²⁹ The clinical examination consisted of the assessment of tooth wear, chipping, or abfraction, masticatory muscle hypertrophy or discomfort, tooth mobility, hypersensitivity, clicking of the temporomandibular joint, and tongue or cheek indentation.³⁰ Before the start of treatment, patients were provided a consultation with an expert in temporomandibular disorders. The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) were clinically applied to determine the presence or absence of any temporomandibular disorder.^{31,32} The erosive status of the maxillary anterior teeth was evaluated with the help of the Anterior Clinical Erosive Classification (ACE).³³ Localized anterior loss of tooth structure, including dentin, with minimum wear of mandibular incisors, relatively intact posterior occlusal surfaces, and absence of temporomandibular disorder were diagnosed from the clinical examinations. According to self-reports and clinical signs, all the patients suffered from bruxism-related occlusal stress. Table 1 shows patient-related factors such as age and gender and provides information about the etiology and classification of tooth wear.

The suggested therapy, approved by the patients, was the sandwich veneer approach in combination with Dahl's concept. Patients received a total of 36 direct palatal RBC veneers on their vital maxillary anterior teeth (Table 1). This method can replace the eroded tooth structure with an additive approach at an increased VDO. The residual posterior interocclusal space is progressively eliminated by the passive eruption of the posterior teeth and the slight intrusion of the anterior teeth, reestablishing the occlusal contacts. A total of 42 lithium disilicate-reinforced pressed ceramic veneers were used on the labial surfaces to improve esthetic appearance and maximally preserve the remaining tooth substance while simultaneously balancing the functional stresses on the anterior dentition (Table 1).

Restorative Procedure

Upper and lower one-stage sandwich vinyl polysiloxane impressions (Variotime Easy Putty and Light Flow, Kulzer, Hanau, Germany) were taken. This was followed by bite registration (Variotime Bite, Kulzer) recorded in centric relation obtained by a leaf gauge. The difference between maximal intercuspation and centric relation was also determined. A facebow was used to transfer the relation between the jaws and temporomandibular joint. The study casts were then mounted on an articulator in centric relation. A wax-up was carried out on the palatal, incisal and labial surfaces of the upper six anterior teeth and additionally on the labial surfaces of those premolars which were affected by the erosive effect. The wax-up was the basis for creating an intraoral mock-up (Structur 2 SC, VOCO, Cuxhaven, Germany), which was used as a visual aid to show the proposed treatment outcome and analyze the esthetic and functional (anterior and canine guidance) changes. A silicone mold (Registrado Clear, VOCO, Cuxhaven, Germany or Variotime Easy Putty, Kulzer, Hanau, Germany) from the diagnostic wax-up was used as a trimmed palatal/incisal stent for the direct RBC build-ups to provide a desirable form for the palatal surface. Before the build-up procedure the teeth were isolated with a rubber dam and the palatal surfaces of the anterior teeth were air abraded with 29 μm Al_2O_3 powder with copious irrigation at 1 mm distance for 15 seconds (Aqua-Care Twin, Velopex, London, UK). Only sharp enamel edges were removed with a diamond bur (No.890LF, Meisinger, Neuss, Germany). To prevent bonding to adjacent teeth, every second tooth was restored at the same time. Teeth were separated with slight interproximal stripping (Sof-Lex Finishing strips, 3M, St. Paul, MN, USA) and isolated interproximally by a Teflon (DuPont de Nemours Inc, Wilmington, Delaware, USA) tape. Regarding the adhesive technique, a two-step etch-and-rinse system (Adper Single Bond, 3M) was used for each restoration. Every second tooth was conditioned with the etch-and-rinse technique by applying 37% phosphoric acid for 20 seconds (Ultra-etch, Ultradent, South Jordan, UT, USA), followed by 20 seconds of rinsing and careful drying. The one-step enamel-dentin adhesive was applied by rubbing the exposed dentin and enamel with a micro-brush soaked in the resin. In order to evaporate the solvent, gentle, 10-second air-drying was carried out, followed by 10 seconds of polymerization with a light-emitting diode (LED) curing unit (LED.D, Woodpecker, Guilin, China; $\lambda = 420\text{--}480$ nm) in standard mode, at an

average tip irradiance of 1450 mW/cm^2 with an 8 mm diameter fiberglass light guide. Nanohybrid RBC (Enamel Plus HRi Bio Function, Micerium SpA, Avegno, Italy; BF2 shade) was applied to the matrix in alternating order; then the matrix was seated on the teeth and the excess material was removed from the labio-incisal aspect, maintaining the planned new length as seen with the help of the stent (Figure 1C). The RBC was light-cured for 40 seconds from an incisal direction. After removal of the stent, the palatal surface was directly light cured for a further 40 seconds to provide a high rate of polymerization. Interproximal excess was removed carefully, without causing bleeding, with a metal interproximal strip (Kerr perforated fine diamond finishing strip, Kerr, Bioggio, Switzerland). The restorative procedure was repeated for the remaining teeth. Once all the teeth had been bonded to the palatal aspect, the labio-incisal surface was covered with one more layer of RBC to provisionally complete the anterior restoration at the desired length until the posterior contacts were re-established. The provisional increase in length with direct RBC provided a functional test for the re-established anterior and canine guidance. Patients were informed about possible inconvenience that could be caused by the posterior disclusion and the color mismatch of the lengthened incisal edge before the final facial veneer fabrication. The finishing-polishing procedure was performed with football, needle shape red fine-grit diamond burs (330F and 890LF, Meisinger, Neuss, Germany) followed by the Enhance/PoGo system (Dentsply Sirona, York, PA, USA) and aluminum oxide strips (Sof-Lex Finishing strips, 3M). Then the occlusion was refined to ensure even, stable contacts on all anterior teeth of equal intensity in centric relation (static occlusion) and guidance provided by the incisors and canines (dynamic occlusion) (Figure 1D). The occlusion was developed according to the following principles: The restored palatal surface and the increased incisal length must be in harmony with the envelope of function and should provide unobstructed movement. The palatal cingulum and slightly flat occlusal stop provide stable contact and centric freedom for the lower incisors. This smooth surface helps minimize future wear of the opposing lower incisors and directs forces down the long axis of the tooth, thereby reducing possible labial tooth movement. During lateral excursion the canine guidance should provide incisal disclusion, which is crucial to avoid future fracture of the restored incisal edges. After the occlusal adjustment, final finishing and polishing were performed by the Enhance/PoGo system

and surface gloss was enhanced by goat hair brush, felt disk/wheel and polishing pastes (Enamel Plus HFO Shiny Finishing Kit, Micerium SpA). Posterior disclusion was measured from a wax record taken postoperatively between the first molar teeth (Table 1). The increase of VDO by the additional palatal layer ranged between 1.0 mm and 2.0 mm.

Two to three days after the palatal restorative procedure, patients were scheduled for an anterior occlusal check-up to control the stable contacts and anterior guidance. The investigators also considered it important to assess patient comfort and to test the phonetic implications of the extensive rehabilitation of the maxillary incisors. For a phonetic test, the “s” sound was used, since it can be influenced by horizontal or vertical volume alterations of the anterior teeth.

Progression of posterior tooth eruption was assessed weekly with an $8 \mu\text{m}$ Shim Stock foil (Arti-Fol, Bausch, Köln, Germany) (Figure 2A-D). After the detection of firm contacts on all posterior teeth (four weeks after palatal build-up for all patients) the procedure for labial ceramic veneer fabrication was begun. The first step was the mock-up preparation based on the initial diagnostic wax-up. As the new position of the occlusal plane and the increased VDO may be slightly different from what was initially planned, the length of the maxillary anterior teeth needed to be reconfirmed during this second mock-up session. Once the patient's agreement with the final shape of the maxillary anterior teeth was obtained, the mock-up guided preparation was performed with the help of a depth-marker diamond cylinder bur (No.834, size 016, cutting depth 0.3 mm; size 021, cutting depth 0.5 mm; Komet, Besigheim, Germany) (Figure 3A). Three shallow orientation grooves were cut through the mock-up RBC and/or enamel on the labial surface. Then the mock-up was removed and the mark on the enamel left by the orientation groove was highlighted with a water-resistant pen (1513 Multimark F permanent, Faber-Castell, Stein, Germany). Round-ended tapered diamond burs (No.868 and 8868, Komet, Besigheim, Germany) were used to remove enamel between the marked grooves and to create definitive contouring and finishing. The color marking was not removed completely, to ensure that no more than the desired enamel was prepared. A supra/paragingival chamfer-like finishing line was prepared at a depth of 0.3 mm; this was extended interproximally as an elbow, involving the labial half of the contact area without breaking the contact. A butt-joint incisal preparation was performed, completely removing the length

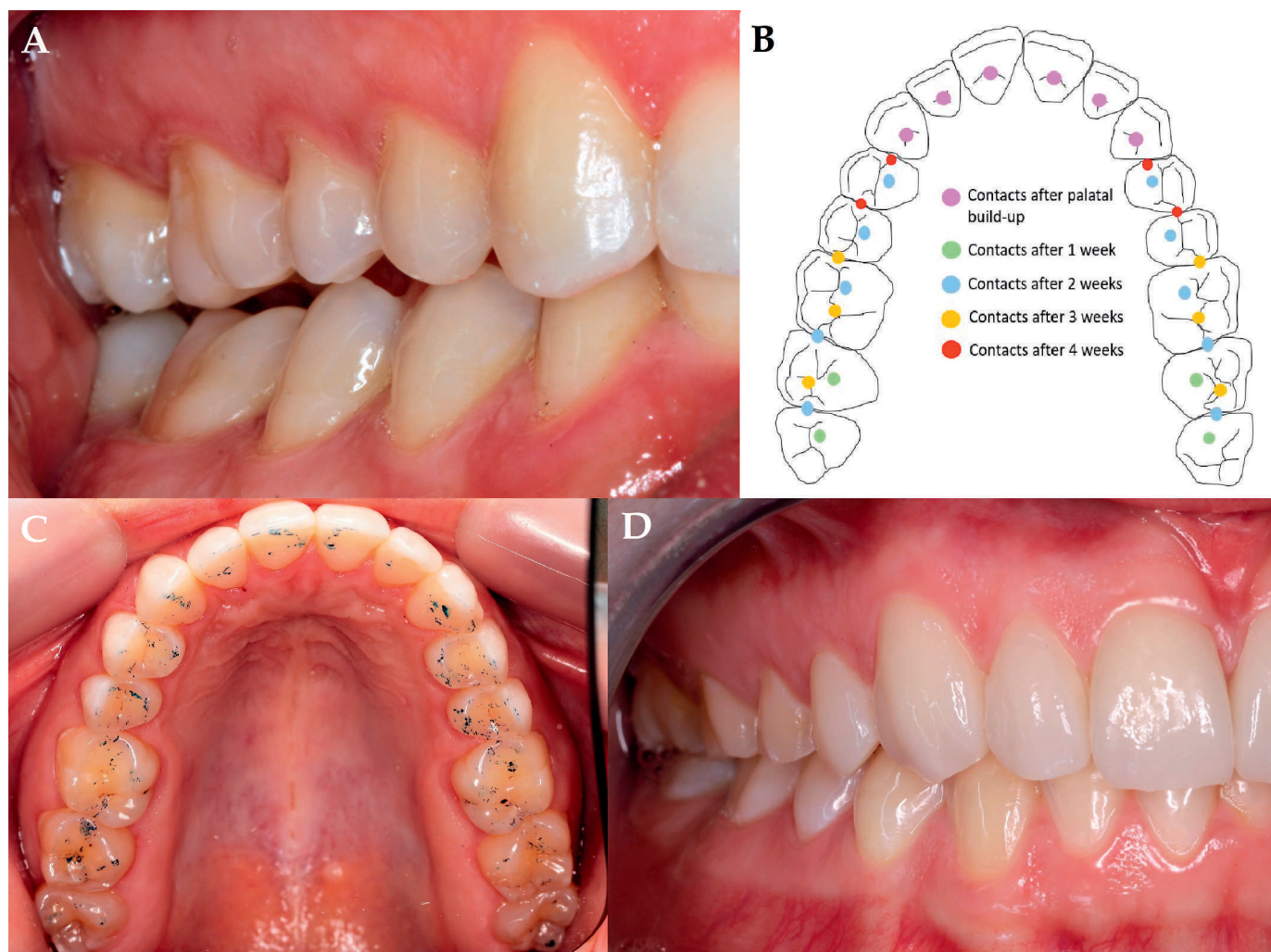


Figure 2. The initially discluded posterior teeth (A) showed a general progression of eruption (B), resulting in firmly re-established posterior contacts (C, D).

added by the provisional RBC build-up, leaving only the original length of the tooth (Figure 3B). The ceramic veneer would later re-establish the final length. The margin was placed in the volume of the palatal RBC (Figure 3B and Figure 4A-C). The prepared surface was polished with abrasive discs (Sof-Lex disks, 3M) and a gingival retraction cord (0, UltraPak, Ultradent) was placed in the sulcus. Upper two-stage and lower one-stage sandwich vinyl polysiloxane impressions (Variotime Easy Putty and Light Flow, Kulzer) were taken. This was followed by bite registration (Variotime Bite, Kulzer) recorded in maximum intercuspation. The shade of the prepared teeth and also the desired shade were selected with the patient's agreement. Provisionals were made with the help of the impression that was fabricated to perform the mock-up. At the next appointment the pressed lithium disilicate-rein-

forced glass-ceramic laminate veneers (GC Initial LiSi Press, GC, Leuven, Belgium) were placed in the mouth to check the fit, and try-in paste (NX3 Nexus Third Generation, Kerr) was used to select the proper shade of the adhesive luting cement (Figure 3C). To achieve durable bond-strength the internal surfaces of the cleaned (Ivoclean, Ivoclar Vivadent, Schaan, Liechtenstein) veneers were etched with 9.5% hydrofluoric acid (Porcelain Etch, Ultradent) for 20 seconds, followed by washing, drying and silane (Silane, Ultradent) application which was left to dry. The luting procedure was started with both cleaned central incisors and progressed bilaterally. The above-described total-etch adhesive procedure was performed on the isolated teeth. To provide accurate fit of the veneer prior to light-curing, the adhesive layer was thinned with air-drying for a minimum of 20 seconds; the removal of excess was



Figure 3. Indirect ceramic veneer fabrication with mock-up guided preparation (A) with butt-joint incisal finishing line (B). Try-in paste helps to select the favorable shade of the adhesive cement (C) to provide a natural appearance for the veneers (D).

aided by the vacuum of the high-volume evacuator tip. A clean microbrush was used to remove the gathered excess at the marginal bevel if the effects of the air-stream and the evacuator needed to be completed. Light-curable adhesive resin cement (NX3 Nexus Third Generation, Kerr) was applied to the veneers, which were then gently seated with finger pressure. Excess cement was removed with a microbrush and a thin modelling brush. The above-mentioned LED curing unit was used to polymerize the cement for 4×20 seconds from labial, mesial, distal, and incisal aspects with 5-second breaks to avoid heat damage to the pulp. The final restorative phase was completed by polishing the marginal areas with a silicone rubber point (PoGo system, Dentsply), after removal of excess set cement with a #12 surgical scalpel (Figure 3D). Restorations were carefully checked for any occlusal interference (static and dynamic). After the one-week follow-up of the

labial veneers, maintenance was provided in each half year.

Clinical Evaluation

From the palatal build-up session, the progression of the compensatory eruption was recorded weekly by the evaluation of the opposing contacts until maximum intercuspation was achieved between each posterior tooth. At the five-week follow-up appointment, the patient's opinion on the esthetic result, post-operative pain or discomfort, and bruxism or TMD-related symptoms were evaluated (Table 2).

In January of 2020, the anterior palatal direct RBC veneers and labial ceramic veneers were evaluated by two examiners independently, using a dental mirror and explorer in accordance with modified USPHS²⁷ criteria (Table 3). Particular attention was paid to the interface between the

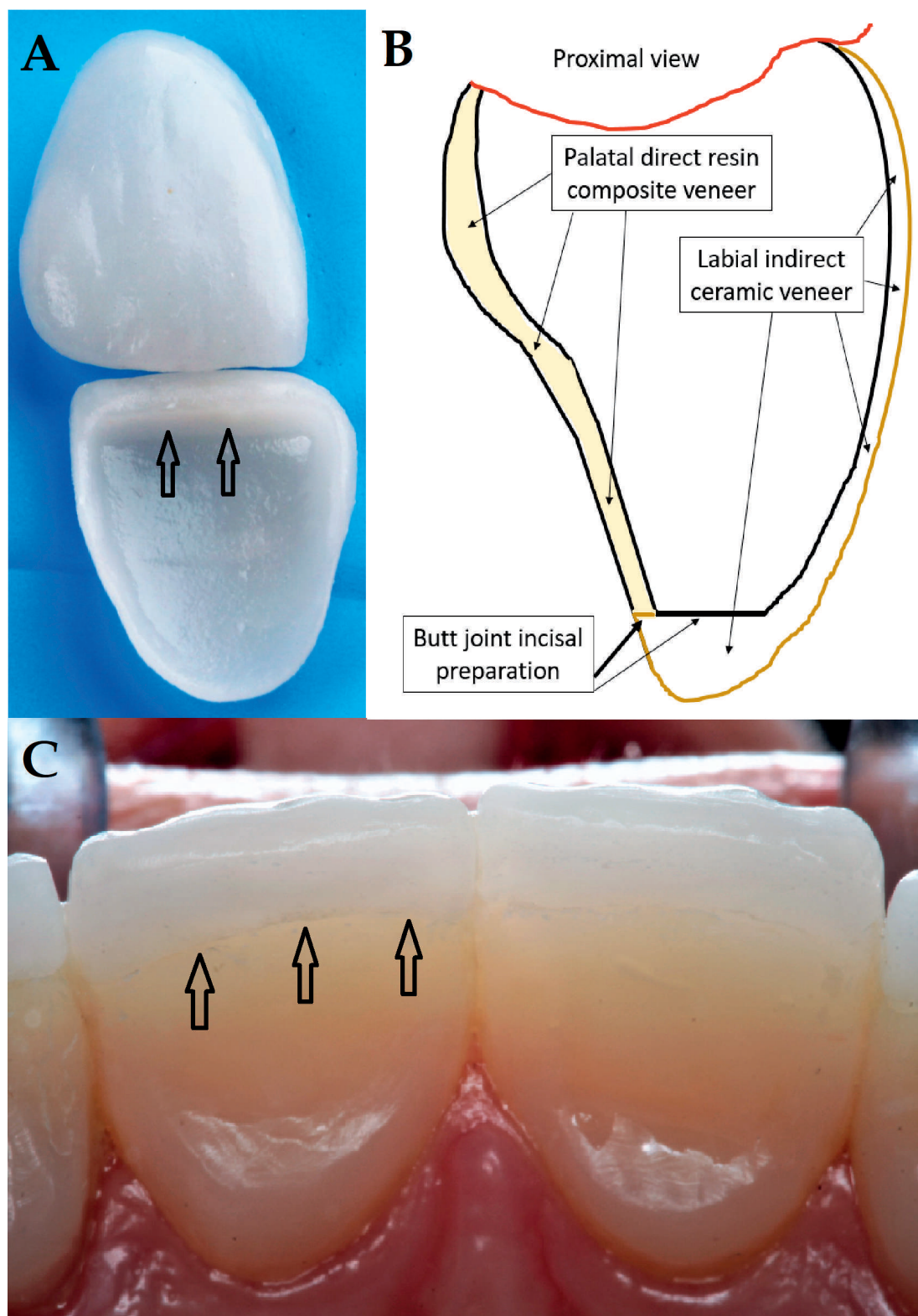


Figure 4. Butt-joint incisal preparation is well seen on the veneer in-surface (arrows) (A), which is seated onto the plane-cut incisal edge including the palatal direct resin composite veneer (B), leading to a palatal-labial veneer interface located on the palatal veneer margin (arrows) (C).

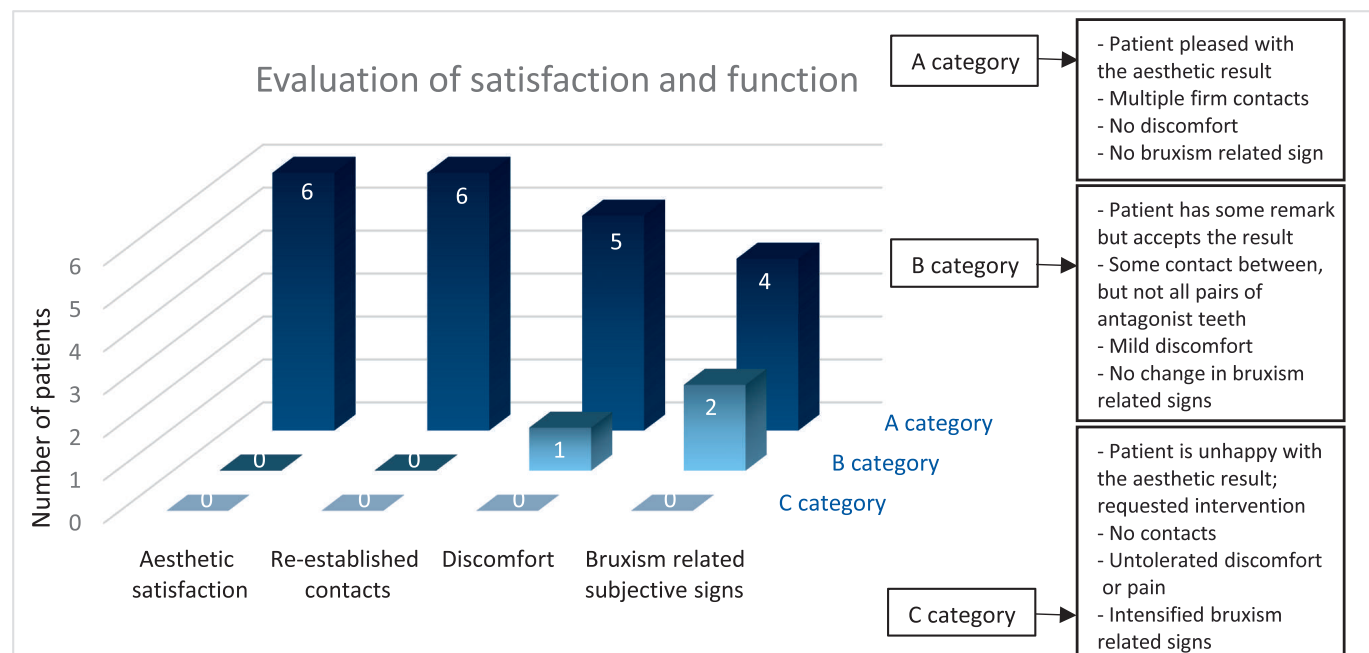


Figure 5. Evaluation of patient satisfaction and function.

palatal direct veneer and the labial indirect veneer in terms of marginal integrity. The time and type of any intervention applied during this maintenance examination were recorded, and patients were questioned again about their esthetic satisfaction, discomfort, and bruxism or TMD-related symptoms.

Data collection and statistical analysis were performed using SPSS for Windows 23.0 (SPSS, Chicago, IL, USA). Because of the low case number, only a descriptive statistical analysis was indicated and only percentages of the respective scores of the USPHS evaluation are given.

RESULTS

In the present observational case-series study a total of 36 direct RBC palatal veneers and 42 labial ceramic veneers were evaluated in six adult patients (mean age: 26.67 at the time of restoration placement) with the diagnosis of localized anterior tooth wear. The follow-up time varied from 20 months to 27 months for palatal veneers and 18 months to 25 months for labial veneers, with a mean observation time of 23.7 and 21.7 months, respectively. The investigators were trained and calibrated before the evaluation. Cohen's kappa statistic showed excellent intraobserver (kappa values of 0.82 and 0.80) and interobserver (kappa value of 0.79) agreement. Figure 5 shows the evaluation of patient satisfaction with the esthetic result, discomfort, re-establishment of posterior

contacts, and bruxism- or TMD-related symptoms at the five-week (from the palatal build-up to one week after the labial veneers were cemented) follow-up appointment. According to one participant's report, discomfort was only present in the first few days after the palatal build-up. Phonetic alterations—in pronouncing the “s” sound—occurred for all patients, but after a short conversation (five- to ten-minute adaptation), it was no longer perceptible. The progression time and order showed almost the same pattern for all participating patients, as shown in Figure 2C. All patients accepted the new VDO well, without showing symptoms of TMD. However, bruxism was still present in two patients (by self-report), although it had not intensified. To decrease the risk of bruxism-related failure, supplemental protection with a night-guard (Michigan-type occlusal appliance) was fabricated for the above-mentioned patients at the five-week check-up (Figure 6). During the subsequent observation period there was no change in esthetic satisfaction, and the stabilized occlusion was reported to be comfortable. Based on the self-reports and clinical examinations of the other four patients, there were no obvious signs of bruxism, despite its being present at the start of treatment. The evaluation, following the USPHS criteria, of the palatal direct RBC and labial ceramic restorations is presented in Figure 7; this evaluation also involved the assessment of the marginal adaptation

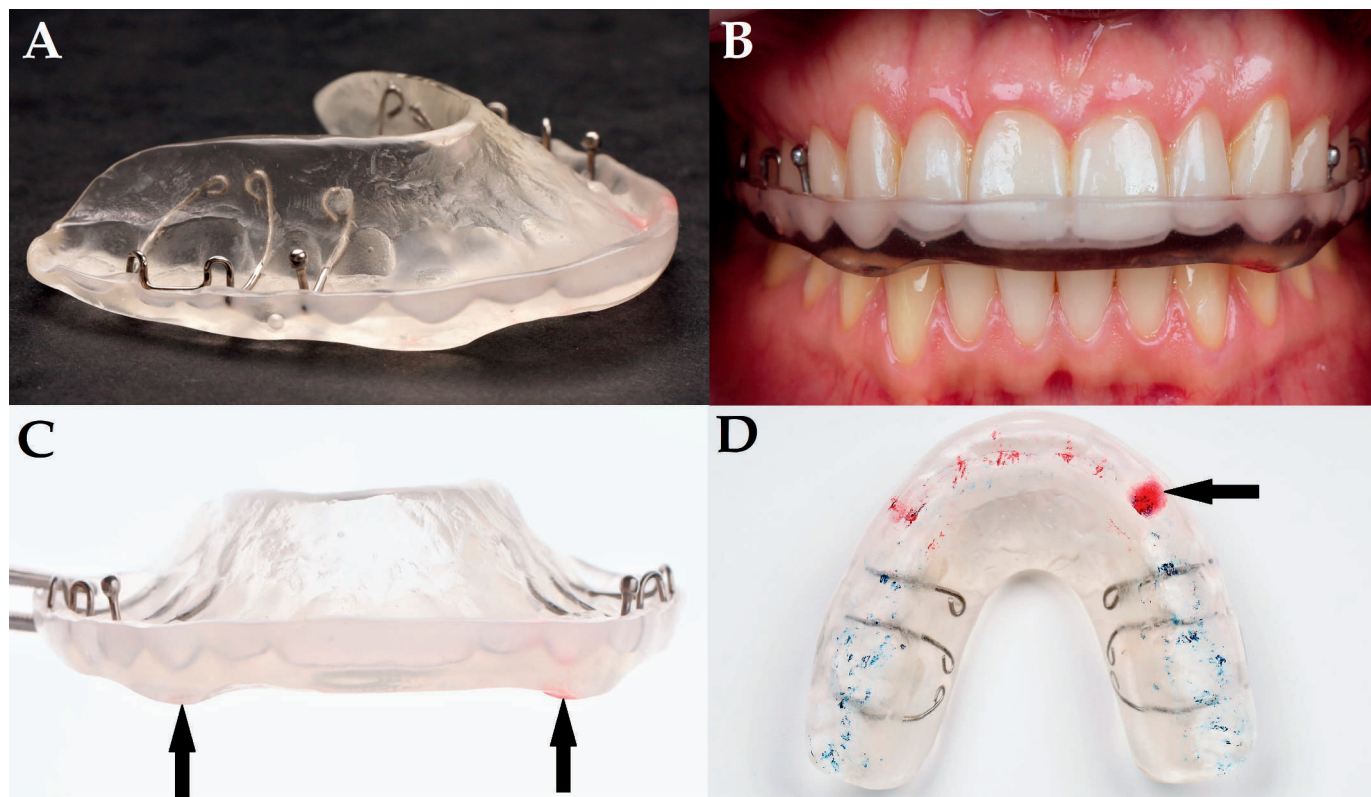


Figure 6. Michigan-type occlusal night-guard splint is fabricated in hard transparent thermoplastic polymer (A) that covers all the maxillary teeth (B) and has flat, simultaneous occlusal centric stops for mandibular teeth. The sloping canine guide plane (C – black arrows) of 1 to 2 mm permits only minimal lateral or protrusive movements under full occlusal contact (D – blue marks) of the lateral splint surface before disclusion occurs. Anterior and canine guidance (D – red marks) provides posterior disclusion. Thin layer of pattern resin was used chair-side to correct the canine guidance on the left side (D – black arrow).

and discoloration of the palatal-labial veneer interface. During the observation period, all the involved teeth were caries-free and remained vital. Of the overall 78 restorations, none were considered unacceptable; the restorations were scored as “alpha” or “bravo” for all items evaluated according to the USPHS criteria. The survival for both types of restoration during this short-term observation period was 100%. Palatal direct restorations had higher rates of small deteriorations for almost every evaluated criterion (Figure 8A), except for anatomic form and color match. Slight wear on the palatal restoration was found to be the most frequent issue (44.4%), followed by slight marginal discoloration (33.3%). Pitted surface roughness was found on all six palatal RBCs (16.6%) in one patient, whereas the texture of all other palatal veneers showed a silky gloss appearance. Slight marginal deterioration was detected in only four palatal veneers (11.1%), which were managed with repolishing. The labial-palatal veneer interface showed mild deterioration in marginal adaptation (16.7%) and marginal discoloration (22.2%). The labial

ceramic veneers showed excellent performance, with slight marginal discoloration detected at only one veneer (Figure 8B).

DISCUSSION

In this prospective, observational case-series study the short-term survival of anterior sandwich veneers performed as Dahl-restorations was evaluated in six patients who suffered from localized anterior erosion and/or attrition. In addition, patient satisfaction, discomfort, progression of posterior tooth eruption, and bruxism-related occlusal stress were also analyzed. Acceptable-to-excellent clinical performance was observed for the sandwich veneers, which consisted of direct palatal RBC and indirect labial ceramic restorations, after a mean observation period of 23.7 and 21.7 months, respectively. However, results and survival analysis should be interpreted with care because, on one hand, the case number is very limited, and on the other, no long-term data are available regarding this sandwich veneer approach. Our primary purpose was not only to describe the outcome of the

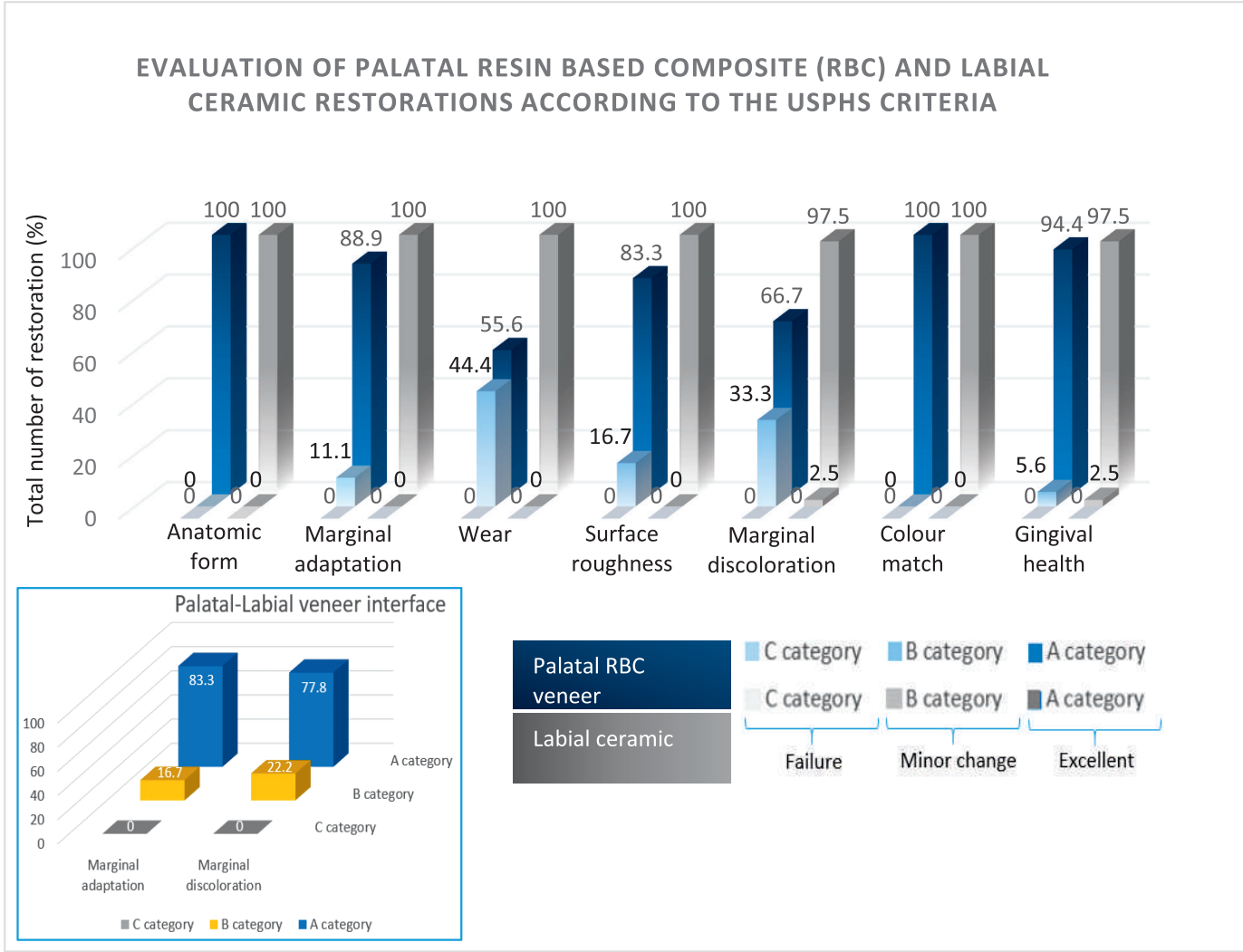


Figure 7. Evaluation of palatal resin-based composite (RBC) and labial ceramic restorations according to the USPHS criteria.

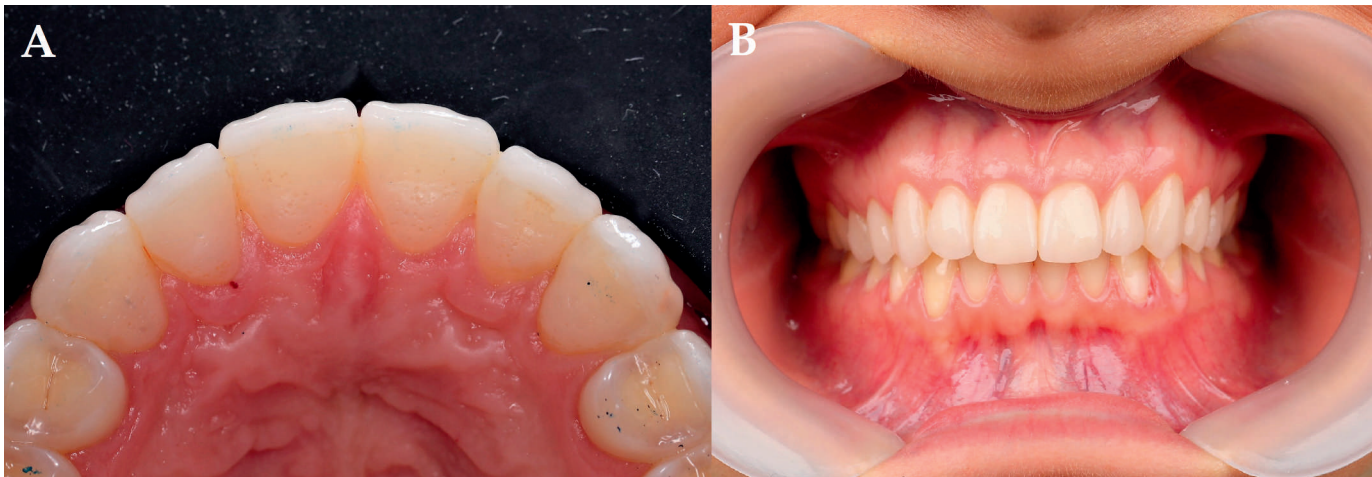


Figure 8. Slight discoloration of the margins and pitted surface roughness of the palatal veneers is detectable (A); however, the labial ceramic veneers were excellent (B) at the 23-month follow-up appointment.

sandwich approach after a short observation period, but also to generate hypotheses that can be tested subsequently, in studies of greater methodological rigor, regarding the survival of this treatment method.

Vailati and Belser introduced a three-step adhesive rehabilitation technique with a minimally invasive sandwich approach that reconstructs the palatal surface first with direct or indirect resin-composite palatal veneers followed by the restoration of the labial aspect with ceramic veneers.^{19,21} This adhesive technique allows preservation of the remaining tooth structure, especially with respect to the intact proximal surface. In a 6-year follow-up study, the authors' subsequent work demonstrated no major detectable failure of sandwich restorations.³⁴ Adhesively bonded ceramic veneers with well-planned surface treatment and cementation protocol represent a highly reliable esthetic and functional long-term treatment option and is supported by several other studies.³⁵⁻³⁸ Due to the low number of cases, our results cannot be supported by statistical analysis, but, in line with the findings of other studies, the labial ceramic veneers performed excellently for each of the examined parameters during the short observation period. The original three-step adhesive rehabilitation technique restores the posterior region with overlays at an increased VDO; however our treatment combined the sandwich approach with the Dahl concept and used palatal veneers as anterior deprogrammers, to be followed by compensatory eruption for the relatively unaffected posterior teeth, reconstructing functional occlusion. This combination of adhesive techniques with the Dahl principle was introduced by Magne and others.²² The Dahl concept allows maximal tooth structure preservation, without the need for posterior overlay restorations, with special attention to healthy molars and premolars. Poyser and others considered the clinically relevant studies related to the Dahl concept and found that the majority of the available literature originated from the United Kingdom.²⁴ Although case-reports are available, there is lack of international uptake of this technique.^{22,24} On the other hand, the quality of the evidence from these studies is medium to low; thus, randomized controlled clinical trials are necessary to provide evidence-based data.²⁴ The original Dahl appliance is a removable, localized palatal bite-raising metal device which can create the necessary space for further full-coverage restorations of damaged anterior teeth.²⁵

To overcome the problems associated with excessive tooth preparation for full coverage crowns, an adhesive double-veneer (palatal metal and labial ceramic) approach was introduced by Bishop and others.³⁹ The improvement and continuous development of adhesive dental materials ensure durable, ultraconservative solutions. The treatment of anterior tooth wear with direct RBC restoration placed in supra-occlusion at an increased VDO was described first by Darbar and Hemmings.⁴⁰ Direct RBC replaces the eroded tooth structure with an additive approach and additionally can be used as an anterior deprogrammer to reposition the mandible in centric relation.^{8,22} Hemmings and others reported an 89.4% success rate of direct resin composite palatal veneers in the treatment of localized anterior tooth wear after 30 month follow-up, and the posterior occlusion was satisfactorily restored after a mean duration of 4.6 months with good patient satisfaction.²⁶ Redman and others, in their short-to-medium term study, confirmed that RBC restorations placed at an increased VDO are viable first-line options to treat anterior tooth wear; the authors concluded, however, that the probability of failure is increased after five years.²⁷ In their medium-term survival study, Gulamali and others concluded that more than 90% of the investigated direct and indirect RBC restorations had exhibited minor or major failure over the preceding ten years and required intervention and maintenance.²⁸ Mechanical limitations of RBCs can lead to marginal fracture and staining, but failures such as bulk fracture, surface roughness and discoloration are uncommon.²⁷ With respect to most deteriorations, a similar tendency was also detected in our study. However, survival of palatal restorations was found to be 100%. A few restorations had minor defects such as surface roughness, marginal discoloration, marginal adaptation, and consequent gingival inflammation after the short observation time. In line with Redman and others, surface roughness in one patient took the form of pitting, suspected to be due to the exposure of tiny voids as the palatal RBC wore and erosive effects softened the external layer.²⁷ In our cases, a nano-hybrid RBC was applied directly to the silicon matrix and pressed to the palatal surface of the tooth. Probably the absence of an intensive condensation phase is the reason for the voids and subsequent surface roughness. In contrast, a clinical study found that the indirectly made palatal veneer had a surprisingly higher incidence of roughness.²⁷ Despite the short observation period, marked small wear facets appeared frequently (USPHS "Bravo" score of 44.4%) on the palatal veneers, especially on

the static occlusal contacts. All six patients had suffered from night or awake grinding before the initiation of treatment; however, only two patients reported continuing bruxism after the palatal build-up. They received night-guard splints to prevent fracture of the veneers. Based on the detected wear facets, it is assumed that some degree of occlusal stress was still present in all patients, but the newly constructed anatomic palatal shape and designed guidance limited parafunctional movements, which were consequently less noticeable to the patients. Although only two of our participants needed night-guard splinting, it is important to emphasize that regular check-ups and maintenance are suggested for timely detection of signs of increased bruxism activity. As an alternative solution to decrease the risk of restoration failure, routine fabrication of a night-guard splint at the end of the restorative treatment could be beneficial. It is considered advantageous to use RBC restorations opposite worn teeth, since RBCs have lower wear resistance than the opposing teeth, and thus the wear rate of the antagonist could be decreased.^{41,42} Further advantages of RBC for Dahl restorations are the maintainability, time- and cost-saving potential, and preservation of healthy tooth structures.⁴³ Moreover, it can protect exposed dentin from further wear, and the reversible nature of the approach may allow repair and an alternative restoration in the future. This technique offers good patient compliance and satisfaction, even when repair is needed. Our patients tolerated the increased VDO and posterior disclusion very well. They were maximally adapted within a short time, as has been reported in other studies as well.^{26,27}

The palatal RBC restorations provided a deliberate occlusal plane and axial loading of the antagonist teeth; therefore no adverse tooth movements were detected on the anterior teeth. The fundamental importance of the reconstructed canine guidance lies in preventing fracture of the labial veneer by separating the upper and lower incisors during lateral movements. Already in the design or mock-up phase, it is necessary to determine the future length of the incisors and align them with the available interincisal space provided by the planned canine guidance. Furthermore, it is assumed that the stable anterior occlusion and guidance facilitate posterior tooth eruption by neuromuscular regulation. Posteruptive tooth movement maintains the position in occlusion by compensation for occlusal tooth wear. Although the actual mechanism of compensatory eruption is unclear, it is supposed

that coordinated forces of the orofacial muscles, under the influence of the central nervous system, are responsible for tooth eruption.⁴⁴ Lund has described that the control of mastication depends on sensory feedback that serves to regulate muscle activity through all stages of mastication.⁴⁵ The individual chewing cycle is divided into three stages: opening, fast-closing, and slow-closing. The last is associated with the increased force required for grinding the bolus. It is speculated that when the posterior region is discluded, as in the case of only anterior contacts and guidance, the slow-closing phase and resultant increased elevator muscle activity are absent; thus the food mastication function is disrupted. The central nervous system receives several kinds of feedback from muscles, periodontal receptors, and touch receptors of the mucosa, and consequently induces bone remodeling and tooth eruption to maintain the physiologic function of the masticatory system. In our study, all subjects had re-established posterior contacts within four weeks. Redman and others found that of 31 patients, 61% had complete and 39% had partial re-establishment of posterior occlusion within two to 18 months, with a mean of seven months.²⁷ It is likely that mandibular repositioning and the youth of our patients contributed to the rapid results observed.

CONCLUSIONS

Within the limitations of this observational case-series, the present authors conclude that adhesively bonded labial ceramic and palatal direct RBC veneer—ie, sandwich veneer—in combination with the Dahl concept is a promising method for treating localized anterior tooth wear. The minimally invasive additive approach and the compensatory eruption of posterior teeth reconstruct esthetics and function with maximal preservation of the remaining tooth structure. Although, mechanical limitations of the direct RBC can lead to wear, marginal breakdown, marginal discoloration, and surface roughness, the maintenance of these palatal restorations is straightforward, with refinishing or with localized repair. There is a high degree of patient satisfaction associated with sandwich restorations. The temporary posterior disclusion is well-tolerated and contacts are re-established within a very short period of time.

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Regulatory Statement

The study protocol was approved by the Regional Research Ethics Committee of the University of Pécs (3410.1/PTE).

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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Color Match of Composite Resin and Remaining Tooth Structure over a Period of 28 Days Using Spectrophotometer—A Randomized Clinical Trial

MMJ Hashir • P Ravishankar • S Dhanapal • AR PradeepKumar

Clinical Relevance

The *in vivo* color match of composite restorations to the remaining tooth structure in Class IV restorations can take up to 28 days. The tooth structure can take 48 hours to 28 days to return to baseline color.

SUMMARY

OBJECTIVES: The tooth and the composite restoration can undergo a change in color after the restorative procedure over time. The purpose of this randomized clinical trial was to evaluate the color difference (ΔE) of two types of composite resins and remaining tooth structure over a period of 28 days.

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METHODS AND MATERIALS: A total of 40 teeth in 30 patients with Class IV caries or EllisClass II fracture in maxillary incisors were randomized into two groups based on the type of composite resin used for restoration: IPS Empress (n=20 teeth) and Filtek Z250 (n=20 teeth). Shade selection was done using a spectrophotometer. Restorations were performed using an anatomical layering technique, and finishing and polishing was done after 48 hours. Baseline spectrophotometer color values for the remaining tooth structure were measured before isolation and for composite restorations, 10 minutes after light cure. Further color evaluations using a spectrophotometer for the remaining tooth structure and composite restorations were done at 48 hours (both before and immediately after finishing and polishing), 7days, 14 days, and 28 days, and color difference (ΔE) from baseline values for remaining tooth structure and restorations and ΔE between remaining tooth structure and restorations were calculated. Descriptive

statistics including mean, standard deviation, and frequencies were computed. Mann-Whitney U test was done to compare spectrophotometer values.

RESULTS: There was a significant reduction in mean color difference (ΔE) from baseline values for both tooth ($p < 0.05$) and composite restoration ($p < 0.05$) at all evaluation periods in both groups. Filtek Z250 showed significantly lower mean ΔE between the tooth and the composite resin restoration than IPS Empress at 48 hours (before and after finishing and polishing), 7 days and 14 days ($p < 0.05$). At the 28 day recall, mean ΔE value of IPS Empress restorations to tooth structure was 3.5, while the mean ΔE value of Filtek Z250 restorations to tooth structure was 3.6, which was not statistically different ($p > 0.05$).

CONCLUSION: The remaining tooth structure as well as the composite restorations showed color changes at all evaluation periods, compared to baseline values in both the composite resin groups. Filtek Z250 exhibited less ΔE with remaining tooth structure at 48 hours, 7 days, and 14 days, when compared to IPS Empress. The composite restorations of both the groups reached clinically acceptable color match (mean $\Delta E < 3.7$) with the remaining tooth structure 28 days after the procedure.

INTRODUCTION

Color matching is critical for success in esthetic dentistry, especially in restorations of polychromatic anterior teeth. Achieving optimal color match of composite resins to tooth structure remains a challenge in patients with high esthetic demands.¹

Tooth color is mainly determined by the optical properties of dentin, and enamel contributes with thickness, translucency, and scattering of light.^{2,3} Color match depends on various optical properties of teeth such as hue, value, and chroma, and chromatic properties of composite resins, including fluorescence and opalescence, light transmission and diffusion, surface roughness, and gloss.⁴⁻⁶

Composite restorations can change in color with time.^{7,8} Most of the studies evaluating the color change of composite restorations were done in laboratory conditions and reported color changes after polymerization, water sorption,¹ accelerated hydrothermal, and photo ageing,^{8,9} and UV light

exposure.¹⁰ Teeth can change color due to dehydration and rehydration.¹¹

Clinically, color change in composites and teeth can be evaluated by color measuring devices like spectrophotometers that provide quantitative information on the magnitude and direction of color differences, and are not affected by ambient light.^{2,12}

So far, only a few clinical studies have evaluated the color match of composite restorations to tooth structure using a spectrophotometer.^{13,14} One published case series¹⁴ evaluated the color changes in composite resin and remaining tooth structure in Class IV restorations and found a clinically acceptable color match at 14 and 28 days follow up. A pilot study by Dietschi and others compared the visual and spectrophotometer methods for evaluating the color match of composite to tooth in Class IV restorations.¹³ Further randomized clinical trials comparing composite resin types are required to evaluate the color match of composite restorations to the tooth structure over time.

The optical properties of resin composites can also be influenced by the size, shape, and composition of filler particles¹⁵ as well as the resin monomers.¹⁶ Clinical data comparing anterior restorations done with nanohybrid/nanofilled composites and microhybrid composites is scarce.¹⁷ Therefore, we formulated a randomized clinical trial to evaluate the color match of two different types of composite resins (microhybrid and nanohybrid) to the remaining tooth structure in Class IV restorations over a time period of 28 days¹⁴ using a spectrophotometer. The null hypothesis evaluated was that color difference (ΔE) between resin composite restorations and remaining tooth structure does not change with time.

METHODS AND MATERIALS

Study Design

This was a randomized clinical trial with equal allocation between two groups. The trial was registered in the National Clinical Trial Registry of India (CTRI/2019/12/022546), and it followed CONSORT (Consolidated Standards of Reporting Trials) guidelines (Figure 1).

Recruitment

Study subjects were recruited from the pool of patients referred to the Department of Conservative Dentistry and Endodontics, Thai Moogambigai Dental College and Hospital from 02.01.2020 to 29.02.2020.

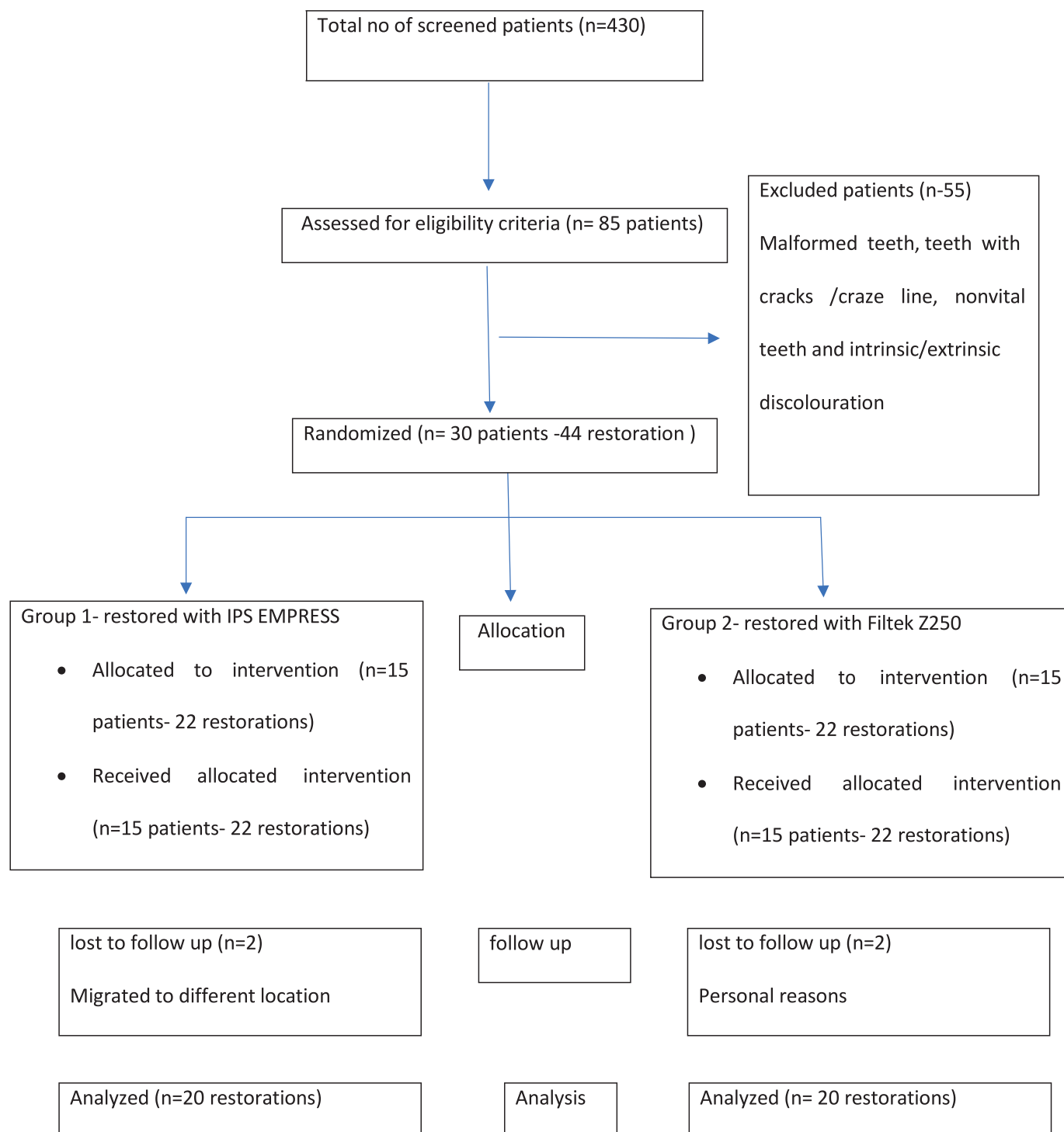


Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram detailing the recruitment and enrollment of the clinical trial.

Inclusion and Exclusion Criteria

The following inclusion criteria were used: Patients of age 15-40 years with Ellis Class II fracture or Class IV dental caries in maxillary incisors who

signed informed consent. Exclusion criteria were malformed teeth, teeth with extrinsic or intrinsic discolorations, teeth with craze lines/cracks, and nonvital teeth.¹³

Sample Size Calculation

An *a priori* analysis was carried out using G power¹⁸ software to calculate the required sample size based upon the given alpha, power, and effect size. A pilot study was conducted among 10 samples each from both groups. The mean color change at 10 minutes, which was 6.3 for IPS Empress group and 4.5 for Filtek Z250 group, was used to calculate the effect size, which was estimated to be 0.40. With alpha error probability of 0.05 and 90% power along with the number of repeated measures (5) and correlation among the repeated measures (0.5), the final sample size was calculated. The resultant sample size was 20 restorations per group.

Random Sequence Generation and Allocation Concealment

A total of 430 patients were screened, and 85 patients with Ellis Class II fracture or Class IV dental caries were identified. Finally, 30 patients requiring a total of 44 restorations (20 plus 2 restorations per group to allow for dropouts) fulfilling the inclusion criteria were included in the study. Seven patients from each group required two restorations. Each tooth requiring a restoration was treated as a separate unit.¹³

Enrolled patients were informed about the study design and clinical procedures involved. Verbal and written consent was obtained. Patients were randomly assigned to one of the two designated treatment groups (Group I - IPS Empress, Group II - Filtek Z250) using a computer-generated randomization method (www.randomization.com, randomization number 16322).

STUDY INTERVENTION

After performing prophylaxis using prophylactic paste and nylon brush, shade evaluation of tooth structure was done using a spectrophotometer (Vita Easyshade V, Vivadent, Brea, CA, USA). Preoperative photographs were made. The enamel shade was selected from the middle-third and the dentin shade was selected from the cervical-third of the tooth, where the enamel is thinner.^{19,20}

All the restorative procedures were performed by a single operator. Isolation was done using a rubber dam and high-volume suction. A bevel was prepared on the enamel margins with a flame-shaped diamond (TR-19, MANI, INC, Tochigi, Japan) at a 45° angle to the external tooth surface to a width of 0.25-2 mm, depending on the amount of tooth structure lost.^{19,21}

The proximal surfaces of adjacent unprepared teeth were protected by using Teflon tape. 37% phosphoric acid etching gel (Total Etch, Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 30 seconds on enamel and 15 seconds on dentin, approximately, 0.5 mm beyond the prepared margins. The etchant was rinsed with water for 5 seconds and dried with blotting paper to avoid overdrying of dentin. A universal adhesive (Adper Single Bond, 3M Oral Care, USA) was rubbed on the etched surfaces with a disposable brush (Dentsply Sirona, Milford, DE, USA) for 20 seconds and air-dried for 5 seconds following the manufacturer's instructions. The adhesive was light cured for 10 seconds with an LED curing unit (Bluephase N, Ivoclar Vivadent).¹⁴

The restoration was performed using the natural layering technique²² using either IPS Empress Direct (Ivoclar Vivadent) or Filtek Z250 (3M Oral Care), based on the allocated group. The composite resin shades were based on the preoperative spectrophotometer readings as previously described. A matrix strip was held palatally, and a layer of enamel shade was placed followed by the dentin shade placed covering the bevel.²³ Finally, labial enamel shade was placed. Each layer was photocured separately for 20 seconds with an LED unit of intensity 1200 mW/cm² (Bluephase N, Ivoclar Vivadent), which was monitored using a radiometer before each restoration. Gross finishing and occlusal adjustments were done for all restorations. The restorations were finished and polished 48 hours after completing the restoration. Proximal surfaces were finished with abrasive strips (3M Oral Care). To remove excess and to reproduce anatomy, flame shaped and tapered carbide burs (Kerr Corporation, Brea, CA) were used. The labial surfaces were finished with sequentially reducing grit size polishing discs (Sof-Lex Pop On, 3M Oral Care). Final polishing was done using a felt disc (Diamond Felt Disc, FGM, Brazil) and diamond polishing paste (Diamond Excel, FGM).¹⁴

Baseline color of the tooth was recorded before isolation, and baseline values of composite restorations were taken 10 minutes after restoration using a spectrophotometer (VITA Easyshade V, Vivadent, Brea, CA, USA). The color of the restorations and remaining tooth structure was measured at different evaluation periods: 1) at 48 hours, before finishing and polishing; 2) at 48 hours, after finishing and polishing; 3) 7 days after completing the restoration; 4) 14 days after completing the

restoration; and 5) 28 days after completion of the restoration.¹⁴ Three consecutive readings of $L^*a^*b^*$ values were recorded and the mean calculated. To standardize the placement of the spectrophotometer on the tooth surface and composite restoration at each time interval, silicone matrix indices (Aquasil Soft Putty, Dentsply) were made with the perforations matching the diameter of the tip of the spectrophotometer.¹⁴ Evaluations were performed with the spectrophotometer and silicone matrix positioned on the tooth without any colored background. An independent and calibrated evaluator, who was not involved in the restorative procedures and blinded to the group assignment, performed all the assessments. Figures 2 and 3 represent preoperative and postoperative photographs of teeth restored with IPS Empress and Filtek Z250, respectively, at various evaluation periods.

The ΔE at each evaluation period (48 hours before and after finishing and polishing, 7 days, 14 days, and 28 days) from baseline was calculated from the $L^*a^*b^*$ values for remaining tooth structure and restoration based on the following equation:²⁴ $(\Delta E) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where $\Delta L^* = L_{\text{Final}} - L_{\text{Initial}}$, $\Delta a^* = a_{\text{Final}} - a_{\text{Initial}}$, and $\Delta b^* = b_{\text{Final}} - b_{\text{Initial}}$. Color difference between the tooth structure and composite restoration was also calculated for each evaluation period.

STATISTICAL ANALYSIS

Statistical analysis of the data was done using Statistical Package for Social Sciences, (SPSS v 21, IBM Corp, Chicago, IL, USA). Descriptive statistics including mean, standard deviation, and frequencies were computed for various clinical parameters. Normality of the data was assessed using Shapiro-Wilk test. Further analysis was done using non-parametric tests, since the data did not follow a normal distribution. Friedman test was used to compare the mean rank differences within the study groups at different time intervals. Mann-Whitney test was used to compare the differences between the groups.

RESULTS

A total of 28 patients with 40 restorations IPS Empress group (n=20) and Filtek Z250 group (n=20) reported for follow up at 48 hours, 7 days, 14 days, and 28 days, while one patient (with two restorations) from each group was lost to follow up, since one had migrated to a different location and the other due to personal reasons.

IPS Empress Group

In the IPS Empress group, mean ΔE values of the remaining tooth structure and the composite restorations showed a significant reduction at all evaluation periods (Table 1). Mean ΔE values of the remaining tooth structure and mean ΔE values of composite restorations were similar at 48 hours (after finishing and polishing) 7, 14, and 28 days ($p>0.05$) (Figure 5).

When evaluating the color difference (ΔE) between remaining tooth structure and composite, mean ΔE values significantly reduced at all evaluation periods to a minimum value of 3.5 at 28 days, which was below the clinically acceptable threshold of 3.7 (Table 3).²⁵

Filtek Z250 Group

In the Filtek Z250 group, mean ΔE values of the remaining tooth structure and composite restorations showed a significant reduction at all evaluation periods (Table 2). Mean ΔE values of the remaining tooth structure and mean ΔE values of composite restorations were significantly different at all the evaluation periods ($p<0.05$) (Figure 5).

When evaluating the color difference (ΔE) between remaining tooth structure and composite restorations, mean ΔE values significantly reduced at all evaluation periods to a minimum value of 3.6 at 28 days, which was below the clinically acceptable threshold of 3.7 (Table 3).²⁵

Post hoc analysis

The mean color difference values (ΔE) between tooth and composite compared at all time points for both IPS Empress and Filtek Z250 were statistically significant ($p<0.05$). Comparison of mean ΔE values of composite restorations at different time points were significant for both types of composite ($p<0.05$). When the mean color difference (ΔE) of tooth structure was compared at different time points, all comparisons were significant ($p<0.05$).

When IPS Empress and Filtek Z250 were compared, Filtek Z250 exhibited significantly less mean color difference (ΔE) between the tooth and composite resin restoration than IPS Empress at 48 hours (before and after finishing and polishing), 7 days, and 14 days ($p<0.05$). At 28 days evaluation, both composite groups showed similar mean color difference (ΔE) between the tooth and composite restoration ($p>0.05$) (Figure 4).



Figure 2. IPS Empress group.

- a) Preoperative
- b) 10 minutes after placement
- c) 48 hours before finishing and polishing
- d) 48 hours after finishing and polishing
- e) 7 days
- f) 14 days
- g) 28 days

DISCUSSION

Color match of composite restorations to teeth can be challenging due to changes in optical properties of the tooth structure¹¹ and composites,^{1,14} following the restorative procedure. *In vitro* studies have reported a clinically acceptable color match of composite resins to tooth structure in Class IV restorations performed by natural layering tech-

niques evaluated using different methods.^{26,27} However, clinical research is necessary, as it may not be possible to directly extrapolate laboratory results to clinical conditions.²⁶ There are only a few clinical studies evaluating the color match of esthetic anterior composite restorations to teeth.^{13,14,25} Therefore, the present *in vivo* randomized clinical trial was done to assess the color change in the



Figure 3. Filtek Z250 group.

- a) Preoperative
- b) 10 minutes after placement
- c) 48 hours before finishing and polishing
- d) 48 hours after finishing and polishing
- e) 7 days
- f) 14 days
- g) 28 days

remaining tooth structure and composite resin and color match of composite resin to the remaining tooth structure using a spectrophotometer over a period of 28 days. Spectrophotometer evaluation can result in a more objective and superior color match between restorations and tooth.^{2,12,28}

Patients evaluated included young adults between 15 and 40 years of age. Older patients were excluded, as teeth can become darker with increas-

ing age due to secondary dentin deposition.²⁹ Seven patients required more than one restoration. Since each tooth was treated as a single unit, and the color difference between the restoration and remaining tooth structure was evaluated, this might not affect the validity of the results.^{13,14} The teeth selected for this study had structural loss not more than the middle-third region, and also the teeth were vital and without any discolorations. Dentin

Table 1: (IPS Empress Group) Mean and Standard Deviation Values of ΔE of the Remaining Tooth Structure and ΔE of Composite Restorations at Different Evaluation Periods Compared with the Baseline Values

Period of Evaluation	Tooth (ΔE)	Composite (ΔE)	p-value
48 h before polishing	5.0 $\pm$ 1.1 <i>a</i> [*]	5.3 $\pm$ 1.0 <i>b</i> [*]	<0.001
48 h after Polishing	4.8 $\pm$ 0.8 <i>a</i> [*]	4.8 $\pm$ 0.6 <i>a</i> [*]	0.601
7 days	4.3 $\pm$ 0.9 <i>a</i> [*]	4.2 $\pm$ 0.7 <i>a</i> [*]	0.562
14 days	3.8 $\pm$ 0.7 <i>a</i> [*]	3.6 $\pm$ 0.4 <i>a</i> [*]	0.333
28 days	3.4 $\pm$ 0.4 <i>a</i> [*]	3.3 $\pm$ 0.3 <i>a</i> [*]	0.378
p-value	<0.001	<0.001	

Different letters denote significant differences between ΔE of tooth and ΔE of composite restorations at various time intervals. ^{*}Denotes significant difference within the groups at various time intervals.

shade was selected from the cervical-third, and enamel shade was selected from the middle-third of the remaining tooth structure^{13,19,20} Anatomical layering technique was performed to match the color of the tooth.^{13,14,26,27}

The present study evaluated mean ΔE values between remaining tooth structure and composite resin at different time periods. The baseline color value of the tooth was recorded before isolation. Baseline color values of composites were recorded 10

minutes after light curing,¹⁴ since the most clinically relevant color changes have been shown to occur immediately after polymerization. Also, polymerized resin composite mock-ups have been recommended to clinically evaluate the color match to tooth structure.^{1,30}

The acceptability threshold of color match between tooth and restorations (ΔE) has been reported to range from 2.6 to 5.6,^{25,28,31} while a literature review has reported that most studies considered the perceptibility threshold (ΔE) as 1.³² In laboratory research, an acceptability threshold (ΔE) below 3.3 has been recommended,³³ while clinical conditions may require a higher acceptability threshold.³⁴ Research to evaluate color match threshold can be affected by the number of observers, methods, and conditions of color matching, disparity between the visually observed region, and the region measured by the instrument and type of color measuring instrument.³⁵ A prospective multicenter study using ceramic specimens in a simulated clinical setting found the 50%:50% acceptability threshold to be 2.7. Also, a recent literature review reported an acceptability threshold of $\Delta E < 2.7$ for a clinical color match between tooth and restoration.³⁶

Previous clinical studies evaluated the tooth-composite color match with different acceptability thresholds.^{13,25} While Dietschi and others¹³ did a

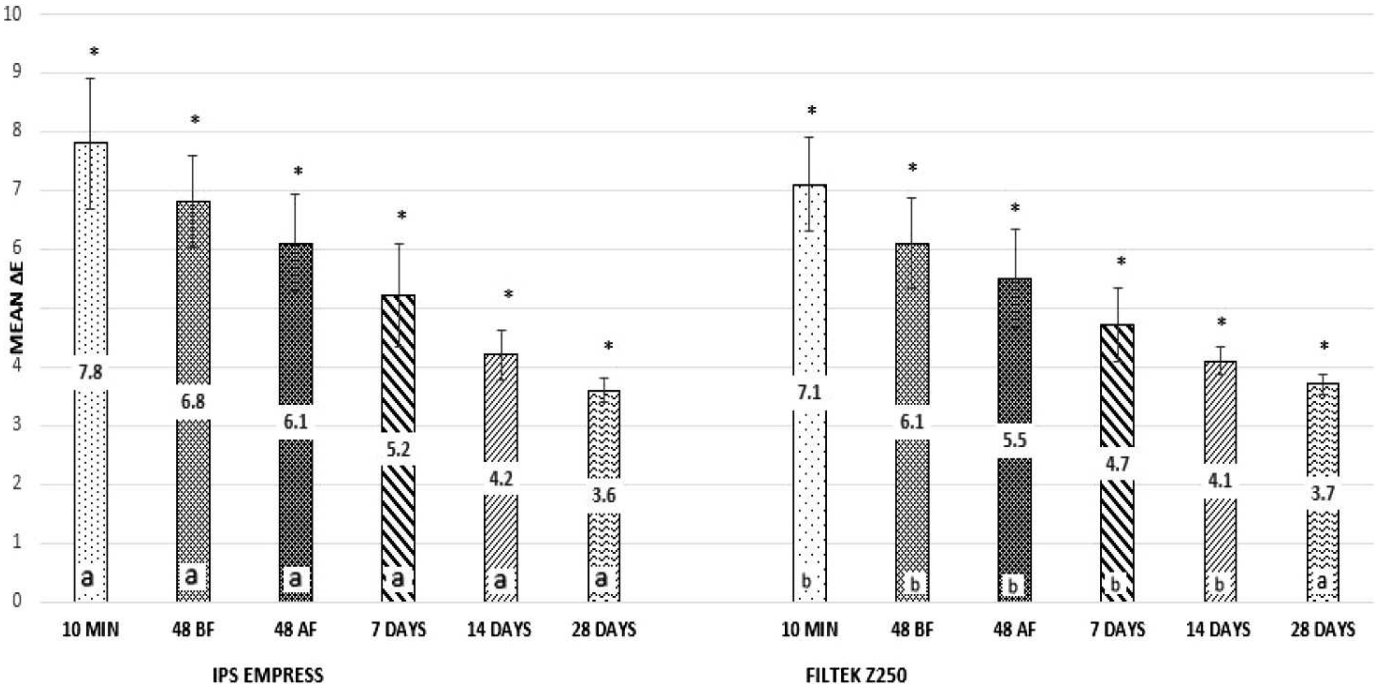


Figure 4. Mean ΔE between tooth and composite. Different letters denote significant difference between the composite groups at corresponding time intervals. ^{*}Denotes significant difference within the composite groups.

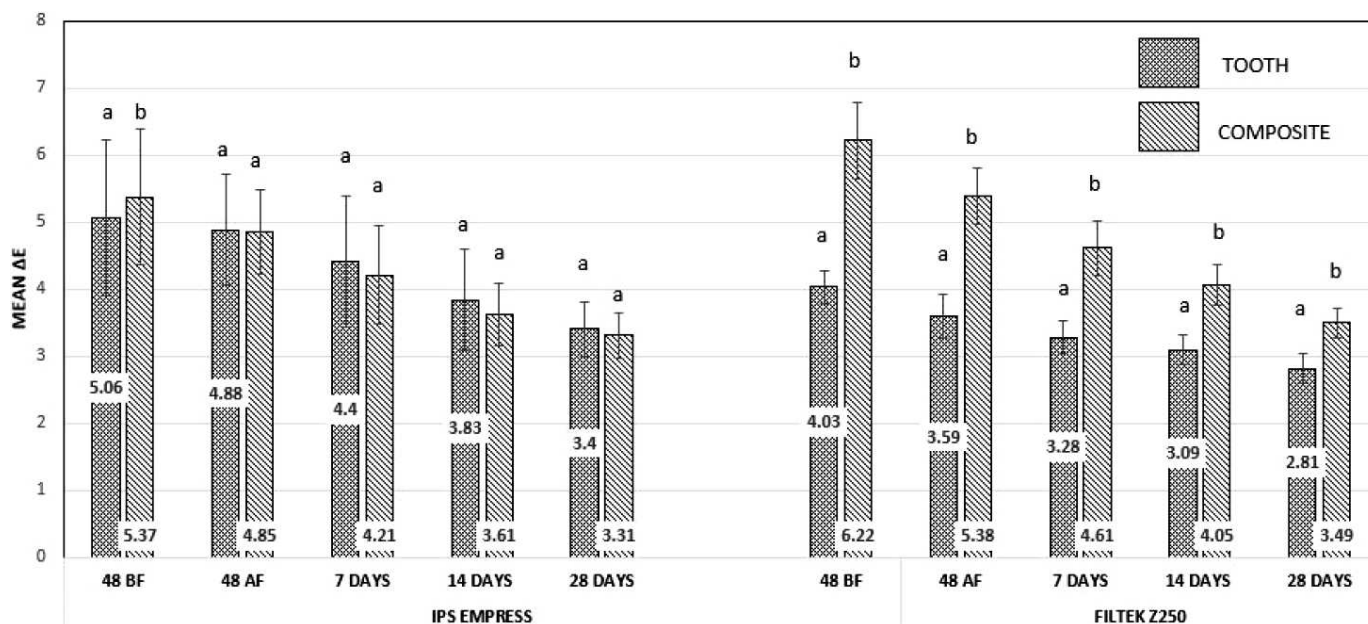


Figure 5. Comparison of ΔE of tooth and ΔE of composite for IPS Empress & Filtek Z250 groups. Different letters denote significant difference between ΔE of tooth and ΔE of composite restorations at corresponding time intervals.

pilot study with 11 patients and reported a mean ΔE of 2 as acceptable, Johnston and Kao have more thoroughly evaluated the color match of composite veneer restorations with adjacent, opposing, or contralateral teeth and recommended a mean acceptability value of 3.7 using a colorimeter.²⁵ Also, Khashayar and others have reported that one-third of clinical studies referred to an acceptability threshold (ΔE) of 3.7.³²

There have been no previously published articles evaluating the color threshold for clinical acceptance of composite restorations using an intraoral spectro-

photometer. Therefore, in the present study, tooth-composite color match was assessed with a clinical acceptability threshold of 3.7,³² and it was found that mean ΔE values in both the groups reached below 3.7 at 28 days.

The thickness and optical properties of the composite layers in restorations can also affect the color match with tooth structure.^{37,38} Hence, in our study, we used a natural layering technique with anatomical proportions of enamel and dentin shade composite resin.

Table 2: (Filtek Z250 Group) Mean and Standard Deviation Values of ΔE of the Remaining Tooth Structure and ΔE of Composite Restorations at Different Evaluation Periods Compared with the Baseline Values

Period of Evaluation	Tooth (ΔE)	Composite (ΔE)	P Value
48 h before polishing	4.0±0.2 a	6.2±0.5 b	<0.001
48 h after Polishing	3.5±0.3 a	5.3±0.4 b	
7 days	3.2±0.2 a	4.6±0.4 b	0.03
14 days	3.0±0.2 a	4.0±0.3 b	0.04
28 days	2.7±0.2 a	3.4±0.2 b	0.02
P value	<0.001	<0.001	

Different letters denote significant differences between ΔE of tooth and ΔE of composite restorations at various time intervals. * Denotes significant difference within the groups at various time intervals.

Table 3: Mean and Standard Deviation of Color Difference (ΔE) Between Remaining Tooth Structure and Composite Restorations of IPS Empress Group and Filtek Z250 Group

Period of Evaluation	IPS Empress	Filtek Z250	P Value
10 min after restoration	7.7±1.1 a	7.1±0.8 b	0.031
48 h before Polishing	6.8±0.7 a	6.1±0.7 b	0.001
48 h after Polishing	6.1±0.8 a	5.4±0.8 b	0.004
7 days	5.1±0.8 a	4.7±0.6 b	0.039
14 days	4.2±0.4 a	4.0±0.2 b	0.001
28 days	3.5±0.2 a	3.6±0.1 a	0.110
P value	<0.001	<0.001	

Different letters denote significant difference between the composite groups at corresponding time intervals. * Denotes significant difference within the composite groups.

The results of the present clinical study showed that a color match was achieved for anterior teeth restored with the microhybrid resin Filtek Z250 and the nanohybrid resin IPS Empress at 28 days. This finding agrees with a meta-analysis done by Maran and others, which could not find any statistical difference in the color match between traditional microhybrid, nanofilled, and nanohybrid posterior composite resin restorations. However, the studies included in that meta-analysis³⁹ analyzed the color match using modified USPHS criteria in posterior teeth, while the present study used the more objective method of color measurement using a spectrophotometer and was done in anterior teeth where the color match is more critical.

Mean ΔE of remaining tooth structure from baseline values significantly reduced from 5.0 (at 48 hours) to 3.4 at 28 days in the IPS Empress group and from 4 (at 48 hours) to 2.7 (28 days) in the Z250 group, as tooth structure dehydrated during the restorative procedure can progressively rehydrate and revert to the original color.^{11,14,40,41}

Composition of the resin matrix, filler loading, filler particle size distribution, and type of photoinitiator may also play a role in color stability.^{15,16,42} Also, it has been demonstrated that the color stability of composites can be affected after exposure to various physicochemical conditions, such as UV radiation, heat, and water or food colorants.⁴³⁻⁴⁸

Composite restorations in both the IPS Empress group and the Z250 group showed a significant reduction in mean color change from baseline values at all evaluation periods to 3.3 and 3.4, respectively, at 28 days. Similar reduction in mean ΔE values of composite restorations at 48 hours, 7 days, 14 days, and 28 days were previously reported by Ruschel and others.¹⁴

Both composite resin groups showed improvement in color match with the remaining tooth structure after finishing and polishing, which is consistent with several studies stating that finishing and polishing can have a significant effect on the color change of composite resins.^{49,50}

In the IPS Empress group, the mean ΔE value of the remaining tooth structure from baseline was not significantly different from the mean ΔE value of composite resins from 7 to 28 days, indicating a similar rate of color change from baseline values. In the Z250 group, significantly different mean ΔE values of remaining tooth structure and composite restorations were found even at 28 days, indicating dissimilar rates of color change. When the color

difference between tooth and composite was compared, Filtek Z250 showed less color difference (mean ΔE) values at 48 hours, 7 days, and 14 days, when compared with IPS Empress. However, the color difference between the tooth and the composite restorations reached below the clinically acceptable threshold of 3.7 at 28 days in both groups, indicating a clinically acceptable color match. Similarly, color match with the remaining tooth structure at 7 days¹³ and 14 days after restoration¹⁴ have been reported.

Our results are not in agreement with a previous *in vitro* study, which demonstrated a clinically acceptable color match of the composite to tooth structure using a spectrophotometer after placing in distilled water for 24 hours.²⁶ However, *in vitro* research may not match clinical conditions. The color difference between the remaining tooth and composite restoration reduced significantly at 48 hours, 7, 14, and 28 days in both groups compared to baseline values. Hence, the null hypothesis was rejected.

The results of our study imply that it can take up to 28 days for color match between composite and remaining tooth structure, even if proper shade selection is done preoperatively using a spectrophotometer. This can be explained on the basis that color selection of the composite was done before dehydration (isolation) of the tooth. However, after the restorative procedure, tooth as well as composite restoration can exhibit color change over time.^{1,11,14} One of the limitations of the present study was that the thickness of the various layers of composite resin could not be standardized. A split-mouth design, which could have better accounted for the within subject factors, was not used due to a paucity of suitable patients willing to take part in this study. Also, individual color coordinates were not analysed independently. To the best of our knowledge, there are no previous clinical studies that have reported color change of composite restorations over a period longer than 28 days using a spectrophotometer. Hence, future studies with longer follow-up periods are needed to assess long-term color changes in composites.

CONCLUSIONS

The remaining tooth structure as well as the composite restorations showed color changes at all evaluation periods, compared to baseline values in both composite resin groups. Filtek Z250 showed less color difference (ΔE) with remaining tooth structure at 48 hours, 7 days, and 14 days, when compared to IPS Empress. The composite restorations of both the groups reached clinically acceptable color match

(mean $\Delta E < 3.7$) with the remaining tooth structure 28 days after the procedure.

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article. This study did not receive any external financial support directed to its development, aside from the author's support and human and facilities' resource.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects' oversight committee guidelines and policies of the Ethics Committee of the Dr MGR Educational and Research Institute. The approval code for this study is 1312002.

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Composite versus Amalgam Restorations Placed in Canadian Dental Schools

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Clinical Relevance

The misalignment between the amounts of time devoted to teaching each restorative material (composite and amalgam) and numbers of posterior restorations placed in Canadian faculty clinics urges revision, to help optimize the learning, training, and clinical outcomes for dental students.

SUMMARY

Objectives: To investigate the latest teaching policies of posterior composite placement versus amalgam and to determine the actual numbers of posterior composites versus amal-

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gam restorations placed in Canadian dental schools, over the years from 2008 to 2018.

Methods: Emails were sent to Chairs/Heads of Restorative Departments and Clinic Directors of all 10 Canadian dental schools to collect data in the forms of: 1) Questionnaire on current teaching policies of posterior composite and amalgam restorations; 2) data entry form to collect the actual numbers of posterior composite and amalgam restorations placed in their clinics.

Results: For the teaching questionnaire, the response rate was 90% (n=9). Seven (78%) of the responding schools reported that they assign 25%-50% of their preclinical restorative teaching time towards posterior composite placement. While, three (33%) of the responding schools allocated 50%-75% of their restorative teaching towards amalgam placement. Data entry response rate was 80% (n=8). Amalgam material was dominant in the restoration distribution from 2008 to 2012. While from 2013 to 2018, resin composite material was dominant in all eight responding schools. Linear regression analysis revealed a significant increasing trend in placing posterior

composites in all the responding schools over time ($p < 0.05$).

Conclusions: Data analysis revealed a clear trend towards an increase of posterior composite restoration placement and a decrease in the number of amalgam restorations placed. However, the teaching time assigned for posterior composite is not aligned with quantity placed. Review and adjustment of time allocated for teaching and training of each material are recommended.

INTRODUCTION

In the last two decades, an ongoing shift towards the use of resin-based composite materials instead of dental amalgam in posterior restorations has been noted. This increase in resin composite use is a result of increased esthetic awareness and apprehension about mercury within the population.^{1,2} A myriad of factors is behind the popularity of resin composite restorations today, such as esthetic properties as well as the micromechanical bonding to tooth structure through adhesive bonding agents.¹ In addition, the improvements in oral hygiene practices and use of fluoride have resulted in many patients with less susceptibility to caries. Therefore, these patients are more likely to benefit from minimally invasive restorative procedures used for tooth-colored resin composite restorations,^{3,4} where the unnecessary removal of sound tooth structure required for amalgam restorations is eliminated.^{1,4} Another reason for the popularity of resin composite restorations is the reduced need for total replacement of failing restorations because in certain cases failing resin composite restorations can be more easily repaired.³⁻⁵ Moreover, studies have shown that the use of resin composite restorations may increase tooth fracture resistance.^{1,3}

Tooth-colored restorations have undergone remarkable and constant progress over recent decades and have revolutionized dental practice. Clinicians and academicians have become more comfortable including resin composites in their practices as well as in the teaching curricula of dental schools.⁶⁻⁸ A Norwegian study showed that resin composite has become the material of choice in posterior restorations in Norway.⁹ In this study, 99% of dentists agreed to using resin composite when treating primary mesio-occluso-distal (MOD) cavities confined to the outer-half of the dentin.⁹⁻¹¹ Similar results were found in other studies internationally. Eklund (2010) looked into trends in dental treatment in the United States (US), showing that patients

received approximately 50% fewer amalgam restorations in 2007 compared to 1992; also the increase in use of resin composite was 50%.¹² More studies in other countries such as the Netherlands, Croatia, France, the US, Finland, Germany, Sweden, Canada, Ireland, and the United Kingdom also reported that the use of amalgam has declined rapidly.¹²⁻¹⁹ A study conducted in Brazil concluded that direct resin composite is the material of choice for posterior restorations.²⁰

With the established increase in clinical placement of posterior composite restorations, there is also a parallel worldwide increase in the teaching of posterior composite.²¹ Many surveys have been conducted to determine the approach in teaching posterior composite in North American dental schools;^{6-8,22,23} A variation in the teaching approaches has been observed, ie, some institutions have adopted the amalgam-free curriculum, others are considering the removal of amalgam from their teaching, while a large number of schools are still in favor of teaching amalgam placement today.²⁴

The latest survey that investigated the teaching of posterior composite placement in North American dental schools concluded that “teaching the placement of posterior resin-based composites in the US and Canadian dental schools has increased, since the previous survey was conducted in 2004-2005, albeit not as much as in other parts of the world.”⁶ None of the previous surveys have looked into the actual numbers of posterior restorations placed by students in North American faculty clinics based on teaching policies.^{6-8,22,23} The present study is the first ever to consider the count of posterior restorations (composite and amalgam) placed in dental schools around North America.

In order to validate teaching policies and quantify current trends of posterior restoration placement in Canada, the objective of this study was twofold: 1) To investigate the latest teaching policies of posterior composite placement versus amalgam in Canadian dental schools, and 2) to determine the actual numbers of all posterior composites versus amalgam restorations placed by undergraduate students in Canadian schools, over the years from 2008 to 2018. The null hypotheses were: 1) There will be no difference in the time allocated for teaching posterior composite and amalgam within Canadian dental schools and 2) There will be no difference in the number of posterior composite and amalgam restorations placed in Canadian dental school clinics.

METHODS AND MATERIALS

An online search was conducted to locate the contact information of the invited schools. Electronic mails were sent to Chairs/Heads of Restorative Departments and Clinic Directors of all 10 Canadian dental schools to collect data in the provided forms. Invitations to participate in the study were sent in early 2019, followed by three reminders with a period of 6 weeks between each reminder.

Questionnaire on Current Teaching Policies of Posterior Resin Composite and Amalgam Restorations

The questionnaire (Supplemental Table 1) consisted of 10 questions in closed-ended format; the invited schools were asked about the percentage of teaching time devoted to each type of restoration and which technique is taught first (composite or amalgam) in preclinical teaching. One of the questions inquired about competency tests conducted for each type of restoration and at what level, ie, preclinical and clinical. Invited schools were asked about the contraindications considered and taught for each type of restoration as well as the differences in cavity design taught for posterior resin composite compared to amalgam preparations. The questionnaire included questions about the matrix system recommended for resin composite restorations, light curing unit used in each school, and whether an etch-and-rinse or self-etch adhesive system is used for bonding posterior composites. The questionnaire also sought information of whether the school teaches the cusp-cap and cusp build-up techniques using resin composite materials, and if bulk-fill composite materials are used for posterior restorations. A data entry form (Supplemental Table 2) was provided to register the actual numbers of posterior composite and amalgam restorations placed by undergraduate students in faculty clinics over 10 years (2008-2018).

Statistical Analysis

Descriptive statistical analysis was performed to summarize the questionnaire results as well as the ratios of posterior composite versus the total number of posterior restorations (posterior composites and amalgam restorations) performed at each responding school. The ratio was calculated as follows:

Ratio of Composite Restorations

$$= \frac{\text{Number of Composite Restorations}}{\text{Number of Total Restorations}}$$

The ratios were calculated for each school separately at each calendar year. Line and bar graphs

were used to visualize year-by-year trends in data. Linear regression analysis was used to determine annual growth rates for total number of posterior restorations, with year being the independent variable. The slope of the regression line represents the growth rate. Chi-square test of independence was used to compare proportions of posterior composite restorations in the first and last year of available data (2008 and 2018 for the most schools). All inferential analysis was performed with level of significance at 0.05. Data was organized in a Microsoft Excel 2016 spreadsheet. Inferential statistical analysis was performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp, Armonk, NY, USA).

RESULTS

Questionnaire Results

Completed questionnaires were received from 9 out of the 10 invited Canadian dental schools, with a response rate of 90%. Responses were received after sending three reminders, with a 6-week period between each reminder.

In regard to posterior composite preclinical teaching, seven (78%) of the responding schools indicated that they assign 25%-50% of their preclinical restorative teaching time towards posterior composite placement. The remaining schools (22%) spend more than 50% of teaching time on the same subject. Over 50% of the responding schools anticipated that more time will be devoted to teaching the placement of posterior composites in the next 5 years. With respect to amalgam preclinical teaching, five (56%) of the responding schools allocated 25%-50% of their restorative teaching towards amalgam placement. While, three (33%) of them indicated that 50%-75% of their preclinical restorative teaching is devoted to teaching amalgam. One school assigned less than 25% of teaching time on the same subject. Only one school anticipated that less time will be allocated to teaching amalgam placement in the next 5 years. Competency tests are found to be indicated for both resin composite and amalgam equally in 67% of the schools (n=6). Only one out of the nine responding schools indicated that competency tests are only required for posterior composite restorations.

Order of Teaching

Eighty nine percent of the responding schools (n=8) teach the amalgam placement techniques before posterior composite techniques in the preclinical courses. While the remaining 11%, which is equiv-

Table 1: *Posterior Restoration Contraindications Taught in Canadian Dental Schools*

Contraindications to Posterior Restoration Placement According to Questionnaire Respondents	Number of Responding Schools	
	Amalgam	Composite
Poor oral hygiene /high caries risk	0	8
Cavity gingival margin on root surface	0	5
Inability to place a rubber dam	0	8
Parafunctional activity (bruxism)	1	2
Tooth acting as removable partial denture abutment	1	3
Patient mercury concerns	8	0
Contact with dissimilar metal	6	0
Pregnant patient	1	1

alent to only one school, is teaching resin composite prior to amalgam.

Contraindications of Posterior Composite and Amalgam Placement

Table 1 lists the contraindications of posterior composite and amalgam placement as taught by responding schools. Almost all the responding schools (n=8) identified “poor oral hygiene and high caries risk” as a contraindication for placing posterior composite restorations, and “patient mercury concerns” for placing amalgam restorations.

Cavity Design Differences

Cavity design differences taught for posterior composite compared to amalgam preparation are shown in Table 2. According to the questionnaire responses, 89% of the responding schools (n=8) taught the “slot-type” cavity preparation for resin composite, whereas two of them (22%) taught the same design for

Table 2: *Cavity Design Differences Taught in Canadian Dental Schools for Posterior Composite Compared to Amalgam*

Cavity Design Feature Taught	Number of Responding Schools	
	Amalgam	Composite
Beveled occlusal margins?	0	1
Beveled gingival margin of proximal box?	3	4
“Slot-type” cavities? (i.e., no occlusal component?)	2	8
Retention grooves in full-scale Class II	7	0
Retention grooves in slot-type Class II	4	0
Reverse curve	8	2

amalgam cavity preparation. Beveling of the proximal gingival margin in composite and amalgam preparation was taught in four and three schools, respectively. Moreover, two schools indicated that they teach full-scale Class II composite preparations featuring “reverse curve.” Whilst eight of the responding schools taught the same feature when preparing for amalgam restoration.

Matrix and Wedge Techniques Used

All participated dental schools (n=9) preferred to teach the “sectional matrix and separating ring” technique when restoring Class II composite restorations; four schools also taught using other techniques like “circumferential matrix and wedges” (n=2) and “circumferential matrix with wedges and separating ring” (n=2).

Curing Lights Used

Seventy eight percent of the responding schools (n=7) reported that the LED curing system is the system of choice in their faculty clinics when curing posterior composite restorations. The use of traditional quartz-halogen light was used in two schools (22%).

Adhesive Systems and Composite Materials

Fifty six percent of responding schools (n=5) taught the students to use only the three-step adhesive system (etch-prime-bond) for bonding posterior composites. While, the remaining schools used a two-step adhesive system only. Fifty six percent of schools surveyed (n=5) did not include teaching of bulk-fill composite materials for posterior restorations; four schools reported that they teach the students using bulk-fill composites.

Sixty seven percent of the schools (n=6) indicated that they teach cusp-capping or cusp-build up techniques using resin composite material.

Posterior Restorations Quantitative Data

The response rate for this part of the study was 80%. Eight schools provided the numbers of posterior restorations (composite and amalgam) placed in their faculty clinics over a 10-year period from 2008 to 2018. A total of 181,791 (101,813 composites and 79,978 amalgams) posterior restorations were performed by Canadian undergraduate students in the eight responding schools over the period from 2008 to 2018 (Figure 1). The placement of amalgam material was dominant in the posterior restoration distribution from 2008 to 2012. While from 2013 to

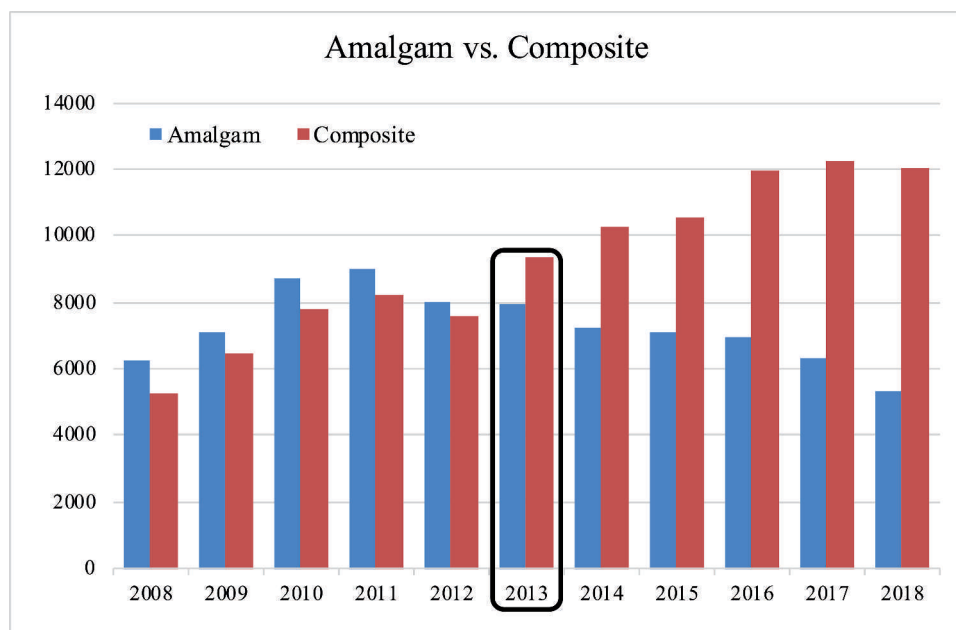


Figure 1. Actual numbers of posterior restorations placed at the eight Canadian university clinics over a 10-year period.

2018, the resin composite material was dominant in the restoration distribution. After 2013, a steady increase in the number of posterior composite restorations placed in all faculty clinics was observed.

Linear regression analysis revealed a significant increasing trend in placing posterior composites in all responding schools over time ($p < 0.05$). Overall proportion of posterior composite restorations has increased from 46% in 2008 to 69% in 2018, while amalgam restorations proportion has decreased from 54% in 2008 to 31% in 2018, representing an annual average increase of 2.5% for posterior composite

placement (Figure 2). When a Chi-square test was performed for each school individually (Figure 3), it showed significantly higher proportions of posterior composite restorations in 2018 compared to the earliest available year. This reveals an overall increasing trend in placement of posterior composites for all Canadian dental schools.

DISCUSSION

Questionnaire Results

One of the objectives of this study was to investigate the latest teaching policies of posterior composite placement versus amalgam in Canadian dental

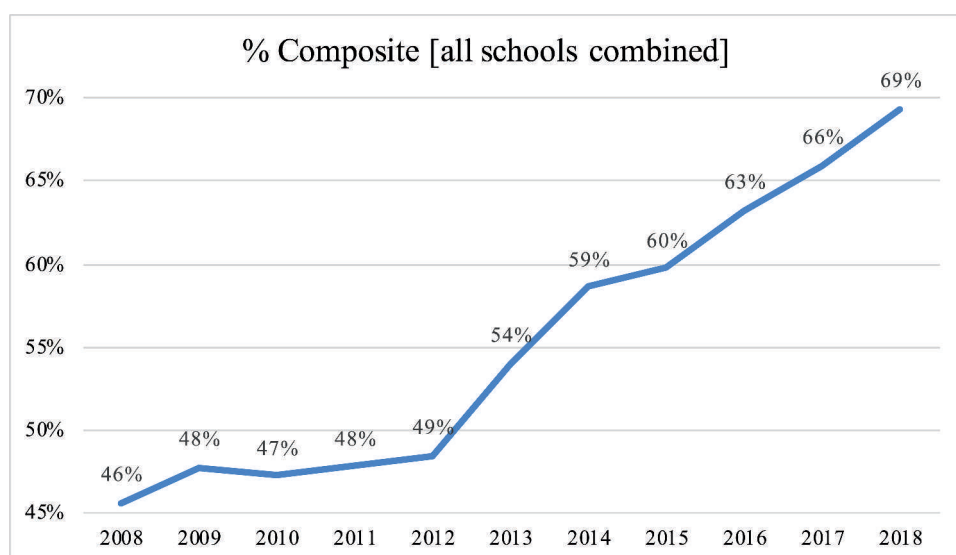


Figure 2. Overall proportion of posterior composite restorations in all eight responding schools.

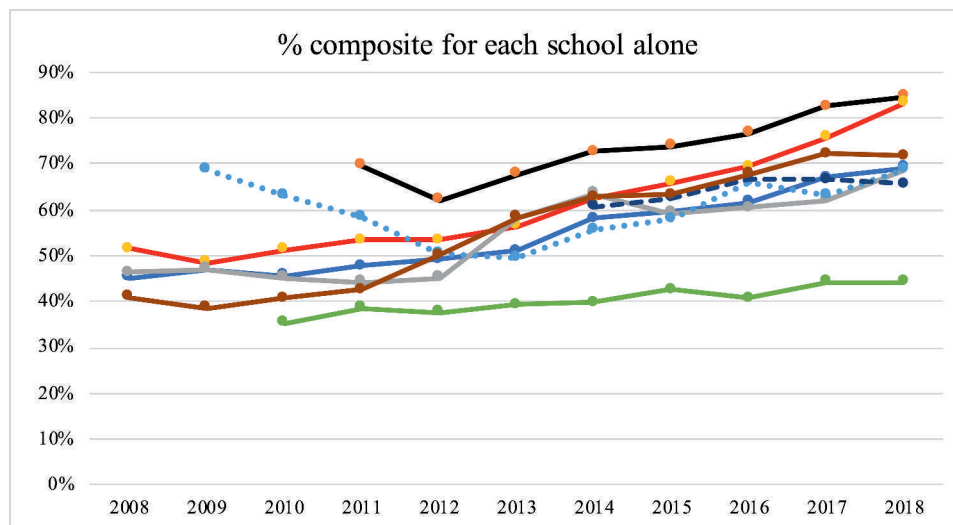


Figure 3. Individual trends of posterior composite placement for the eight responding schools.

schools. The findings of the current questionnaire indicate that the time assigned for teaching posterior composite in Canadian dental schools has not changed since 2005. Most (78%) of the responding schools allocated 25%-50% of their restorative teaching towards posterior composite placement, rejecting the first null hypothesis. Surprisingly, this was the same proportion (25%-50%) assigned for teaching posterior composite in Canadian dental schools, as reported by McComb in 2005.²² On the other hand, Lynch and others reported that the time devoted to teaching posterior composite was found to match the time devoted to teaching amalgam in UK and Ireland dental schools in 2010.²⁵ Five years later, the average percentage of time allocated to teach posterior composite was increased to 36% (range: 10%-75%) and decreased to 25% (range: 10%-45%) for amalgam in 2015,²⁶ meaning that there was a transitional increase in the time percentage allocated to teach posterior composite placement with time in UK and Ireland schools.

In this present study, the teaching of amalgam placement was found to precede the teaching of posterior composite in most schools surveyed (89%). This is unlike in the majority of Japan and United Kingdom dental schools, where teaching the placement of posterior composites is before teaching amalgam placement since 2005.^{2,27,28} The rationale for preceding resin composite teaching to amalgam is that it is easier for dental students to base their approach to restorative dentistry on a preventive, minimally invasive approach to the treatment of caries, rather than on invasive and mechanical retentive principles that the students can transition to, gradually at a later stage.⁶

In the present study, competency tests are indicated for both resin composite and amalgam equally in 67% of the schools (n=6) compared to five schools in the Canadian study 15 years ago.²² An exam of competency is a tool of assessment that has a critical role to evaluate the efficiency of provided education and whether the students have achieved the expected learning outcomes. Preclinical competency tests are meant to simulate real clinical scenarios to be considered valid. As mentioned above, only six schools (67%) currently mandate competency tests in their teaching curricula for both types of restorations, resin composite, and amalgam. However, successful dental education requires both teaching and assessment tools to be paralleled with clinical situations.²⁹

Regarding contraindications for posterior restorations placement, almost all responding schools (n=8) identified "poor oral hygiene and high caries risk" as well as "inability to place a rubber dam" as contraindications for placing posterior composite restorations. And when placing amalgam restorations, responding schools (n=8) identified "patient mercury concerns" as a contraindication in the present study as well as in the previous one (2005).²² These results are also similar to studies in Oceania, Japan, Spain, UK, and Ireland, where rubber dam was preferred in the most cases.^{2,26,30,31} Considering that placement of resin composite material is technique-sensitive by nature, rubber dam placement is crucial to avoid moisture contamination and to ensure successful bonding.³² Moreover, although there is no scientific evidence to support that the release of low levels of mercury vapor associated with dental amalgam restorations are hazardous, almost all schools in the current

study and the previous one (2005) identified “patient mercury concerns” as a contraindication for placing amalgam restorations.³³

Five out of nine responding schools (55%) identified “cavity gingival margin on root surface” as a contraindication to the placement of resin composite in posterior teeth. On the other hand, all responding schools (n=8) from the previous Canadian study taught not to place composite when the cavity gingival margin is on root structure.²² Comparatively, this finding “considering subgingival margin is contraindication to place posterior composites” was only regarded by 9% of dental schools in Austria, Germany, and Switzerland.³⁴ Advances in bonding systems and techniques have resulted in reduced polymerization shrinkage, microleakage, and recurrent caries in posterior composite restorations, which can explain a shift towards higher acceptability for placement of posterior composite in cavities with root and subgingival margins.³⁵

Additionally, in the present study, only two schools considered “parafunctional activity (bruxism)” as a contraindication to restore posterior dentition with resin composite materials, compared to six schools in the previous study (2005).²² The reasons why some schools avoid teaching the placement of posterior composite in bruxing patients is the fear of restoration fracture and wear.³⁶ According to Ferracane (2011), wear is considered to be a lesser problem for current materials as compared to those that were the standard of care a decade ago, in large part due to refinement in the size of the reinforcing fillers that significantly reduced the magnitude of abrasive wear.³⁶ In addition, resin composite material properties have improved to simulate the physical characteristics of enamel and dentin, alongside with growing evidence that supports the placement of composite in posterior teeth.³⁷ This resulted in a substantial increase in the placement of posterior composite restorations even for patients with bruxism or heavy occlusion.^{27,37} Another point to consider is that bruxism diagnosis is not easy, and the criteria for diagnosis are not well-established, as reported by Demarco and others.⁴ They also concluded that no validated criteria to assess bruxing habits, either for grinding or clenching, are yet available. Thus, conclusions regarding a direct relationship between bruxism and restoration failure are still not possible.⁴

In relation to preparation techniques of posterior restorations, 44% of the responding schools (n=4) are still teaching the beveling of the proximal gingival margin in resin composite preparation. In compari-

son, beveling the proximal box margin was taught by 45% of Japanese schools,²⁸ by 60% of Spanish schools,³¹ and by 40% of dental schools in Oceania.³⁰ Beveling the gingival margin of proximal box could challenge the operator to achieve good marginal adaptation and compromise marginal integrity;³ the beveling technique is required when placing anterior composite restorations but not posterior composites.³ Based on the findings of this study, it seems that there is no consensus on preparation techniques for posterior composites among Canadian schools, possibly due to the lack of well-established restorative guidelines in North America.

With respect to the teaching of bulk-fill composite materials when restoring posterior teeth, interestingly, four schools (44%) reported that they do include bulk-fill composites in their restorative curriculum. It is fair to mention that placement of bulk-fill materials is less time consuming and can possibly be polymerized adequately according to Hickey and others.³⁸

Posterior Restorations Quantitative Data

The analysis of data given by the eight responding schools rejects the second null hypothesis, as it revealed that resin composite material surpassed amalgam in the distribution of posterior restorations for the past 5 years (2013-2018) (Figure 1). The placement of posterior composite restorations increased by an average rate of 2.5% every year (Figure 2). A growing transition towards the placement of posterior composite restorations and a continuous reduction in amalgam placement was noted since 2008 (6236 amalgam restorations and 5233 composite restorations were placed in Canadian school clinics in 2008). An Israeli study also noted similar observations in 2018.³⁹ The total number of posterior restorations placed in the past 10 years (2008-2018) was 181,791 (101,813 composites and 79,978 amalgams), asserting that composite material is the predominant posterior restoration material in the Canadian school clinics. It is noteworthy that the number of posterior composites exceeded the number of amalgam restorations in the period from 2013 to 2018, marking the year of 2013 as a turning point. The turning point indicates the starting time when posterior composite restorations were placed more than amalgam restorations.

The clear trend that favors resin composite material since 2013 (Figure 1) can be justified by plenty of reasons. Resin composite esthetic and adhesive properties as well as the controversy over

amalgam safety are likely to be the most popular reasons. Other factors that could have led to such trend are advances in resin composite material and armamentarium, patients, and clinical instructors. For example, creating proximal contact in an occlusoproximal restoration was a real problem before 2008, until sectional matrix systems were introduced, and provided more anatomical and stronger contact areas than a circumferential matrix.⁴⁰ Alongside the continued development of new resin composite materials, like bulk-fill that can be polymerized adequately and more quickly,³⁸ patient preference of restorative material also can play a major role; resin composite esthetic properties can be very appealing to patients, as well as the fear of mercury in amalgam allowed the increase of patients' demand towards resin composite material. The increased number of posterior composite restorations in faculty clinics during the last 5 years of data collection (2013-2018) could indicate that clinical instructors favored using resin composite over amalgam in clinical courses. Clinical instructor preference for resin composite material could be due to gained confidence in posterior composite materials and their several years of clinical experience that led to mastering resin composite placement techniques.

Amalgam material has an undeniable long-standing history of service in dentistry for over 150 years. Amalgam has been the material of choice for many decades due to its strength, durability, resistance to heavy occlusal loads, and the ability to be very effective in otherwise challenging scenarios such as subgingival margins, poor oral hygiene, and moisture control. However, amalgam cavity preparation requires unnecessary removal of sound tooth structure to ensure sufficient resistance and retention forms, which can be avoided with the use of composite material.¹

Principles of posterior composite cavity design depend on minimally invasive techniques that allow micromechanical bonding to the tooth structure without sacrificing sound tissue to achieve resistance and retention forms.¹ The advantage of minimally invasive approach when restoring posterior teeth is by far the most important reason behind the substantial increase in the posterior composites placement in the last 5 years, as shown in Figure 1 (in 2018 alone, 12,033 posterior composites and 5320 amalgam restorations were placed in Canadian schools clinics).^{21,24} Although the overall proportion of posterior composite restorations has increased from 46% in 2008 to 69% in 2018 (Figure 2), our

questionnaire results revealed that in the majority of responding schools (78%, n=7) the restorative teaching devoted to resin composite can be as low as 25%. Only two schools allocated more than 50% of their restorative teaching towards posterior composite placement. The same disconnection was also found in the data provided about amalgam material. Overall proportion of amalgam restorations has dropped from 54% in 2008 to 31% 2018 (Figure 2), yet there are seven Canadian schools who are allocating the same percentage (25%-50%) of the restorative teaching towards resin composite and amalgam equally. The misalignment between the amounts of time assigned to teaching each restorative material and numbers of posterior restorations placed in faculty clinics urges revision of teaching policies.

Restorative teaching should be dynamic and able to renovate over time to meet the requirements of the evolving world of dentistry. Graduating students should be the best prepared to match the expectations of modern dentistry, since students who are graduating in 2018 are expected to practice dentistry till 2050 or even more. Decision makers are responsible to ensure that graduating dentists are confident and ready to address the requirements of modern practice.

CONCLUSIONS

The results of this study quantified a definite trend towards an increase of posterior composite restorations placed in Canadian dental schools in the years from 2008 to 2018. This increase was not paralleled by a similar increase in the time allocated for preclinical teaching. A similar opposite trend towards a decrease in the number of amalgam restorations placed was also observed in all Canadian dental schools. Based on the findings of this study, it is suggested that:

- The time devoted for teaching each restorative material in preclinical courses should be revised and adjusted.
- A national guideline should be developed to help overcome inconsistencies in teaching policies among Canadian schools.

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Regulatory Statement

The author represents that the study was performed in compliance with author's institution's appropriate policies related to the use of animal and/or human subjects and human-derived material.

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

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Biodentine or Mineral Trioxide Aggregate as Direct Pulp Capping Material in Mature Permanent Teeth with Carious Exposure? A Systematic Review and Meta-analysis

Sara Alsubait • Fahd Aljarbou

Clinical Relevance

Mineral trioxide aggregate (MTA) and Biodentine demonstrated high success rates when used as direct pulp capping (DPC) agents in mature permanent teeth with carious exposure. This meta-analysis conducted from clinical studies reveals that both the materials have comparable clinical outcomes.

SUMMARY

Objective: To evaluate the success rate of direct pulp capping (DPC) with Biodentine in mature permanent teeth with carious vital pulp exposure compared to that of DPC with mineral trioxide aggregate (MTA) by means of a systematic review and meta-analysis.

Data Sources: The two authors searched independently the literature published through July 31, 2020, in five electronic databases (PubMed, the Cochrane Central Register of Controlled Trials, Web of Science Core Collec-

tion, the Wiley Online Library, and the SCOPUS database).

Study Selection: The research protocol was previously registered in the PROSPERO database (CRD42020192511). Clinical studies that met the inclusion criteria were chosen and independently screened by the authors.

Data Extraction: A custom-designed spreadsheet was used to extract the data. The quality of each study was evaluated by means of the revised Cochrane risk of bias (ROB) tool or the ROB of nonrandomized studies of interventions tool.

Data Synthesis: Three randomized controlled trials and one retrospective study met the inclusion criteria. Only one study had a high risk of bias. The included studies reported data on a total of 95 participants with an age range of 8-51 years. No significant difference was observed in the overall treatment outcome when comparing Biodentine with MTA (Risk

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ratio=1.00, 95% confidence interval [0.93-1.07], $p=1.00$).

Conclusions: Biodentine had comparable clinical and radiographic success to that of MTA when used as a DPC agent in mature permanent teeth with carious vital pulp exposure. Additional high-quality studies are needed.

INTRODUCTION

Dental caries is a chronic, infectious, transmissible disease affecting the primary and permanent teeth.¹ Clinically, carious lesions have a variant appearance, ranging from microscopic incipient demineralization to cavitated lesions.² Therefore, different treatments can be proposed, ranging from non-restorative treatment in the early stages to complex restorative treatment for large carious defects.^{3,4} During treatment, efforts should be directed toward tooth preservation as much as possible.⁵

Vital pulp therapy (VPT) aims to preserve the dental pulp by placing a biocompatible capping material over the thin layer of remaining dentin (indirect pulp capping), small pulp exposure [direct pulp capping (DPC)], partially removed coronal pulp (partial pulpotomy), or fully removed coronal pulp (full pulpotomy). In primary teeth, pulp capping following carious pulp exposure is not recommended by the American Academy of Pediatric Dentistry.⁶ Furthermore, the results of published trials comparing different dental materials as pulp capping agents in primary teeth were inconclusive.⁷ In permanent teeth, VPT has a good success rate if the pulp is not irreversibly inflamed, the hemorrhage is well controlled, and a good seal with a biocompatible capping material is obtained.⁸ Recent evidence has shown successful outcomes of VPT, even when performed on cases with irreversible pulpitis.⁹

Direct pulp capping (DPC) has a long history of clinical use after carious or traumatic pulp exposure. For several decades, calcium hydroxide [$\text{Ca}(\text{OH})_2$] was the material of choice for DPC.¹⁰ However, $\text{Ca}(\text{OH})_2$ results in incomplete dentinal bridge formation with tunnel defects.¹¹ Since the introduction of mineral trioxide aggregate (MTA) in the early 1990s, it immediately attracted interest as a pulp capping agent. MTA can form a thick dentin bridge that is continuous with the adjacent dentin.¹² Mente and others¹³ reported that the success rate of DPC with $\text{Ca}(\text{OH})_2$ is 59% and that with MTA is 80.5%. However, MTA has several drawbacks, including the ability to discolor the tooth structure, difficulties in handling, and an extended setting time.¹⁴ Bioden-

tine (Septodont Ltd, Saint Maur des Fausse's, France) is a newer calcium silicate cement that was developed to overcome the drawbacks of older cements, such as MTA.¹⁵ When used as a pulp capping material, Biodentine causes the formation of a continuous dentin bridge that is similar to the one formed by MTA.¹⁶ Multiple studies have reported that VPT using Biodentine is a successful treatment option for mature permanent teeth.¹⁷⁻²²

In a recent meta-analysis comparing Biodentine with MTA on DPC success rate to permanent teeth, it was reported that there is no statistically significant difference between MTA and Biodentine.²³ However, studies with immature permanent teeth were included in their analysis.²³ In a more recent meta-analysis, the influence of different pulp capping materials on DPC success rates was evaluated.²⁴ It was concluded that MTA had better success than $\text{Ca}(\text{OH})_2$, and there was no difference in success rates between MTA and Biodentine. The present study focused on comparisons between MTA and Biodentine, when used for DPC in mature permanent teeth. Therefore, the aim of this systematic review and meta-analysis was to evaluate the success rate of DPC in mature permanent teeth with carious pulp exposure using Biodentine compared to MTA.

METHODS

The current systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P) guidelines.²⁵ The protocol was registered on PROSPERO (Registration Number: CRD42020192511).

Developing the research questions

The PICO approach (**P**opulation, **I**ntervention, **C**omparison, and **O**utcome) was used for formulating the following research question: *In a mature permanent tooth with carious exposure and vital pulp, what is the success rate of using Biodentine as a DPC agent in comparison to MTA?*

Search methodology

An electronic search of PubMed, Cochrane Central Register of Controlled Trials, Web of Science Core Collection, Wiley Online Library, and SCOPUS databases was conducted using the following search terms: permanent dentition, mature teeth, dental caries, dental pulp capping, vital pulp therapy (VPT), DPC, tricalcium silicate, MTA, Biodentine,

Table 1: Search Strategy through PubMed		
Number	Search Strategy	Results
1	Dental caries	58,865
2	Permanent dentition OR "mature teeth"	6223
3	Dental pulp capping OR "vital pulp therapy (VPT)" OR "direct pulp capping (DPC)"	2728
4	Tricalcium silicate OR mineral trioxide aggregate OR "MTA" OR Biodentine	10604
5	Treatment outcome OR "success rate" OR prognosis OR survival rate	2,264,290
6	#1 and #2 and #3 and #4 and #5	10

success rate, prognosis, treatment outcome, and survival rate. Table 1 displays an example of the electronic search strategy that was conducted through PubMed. The last search was performed on July 3, 2020.

Eligibility criteria

Initially, titles and abstracts of the papers were evaluated to remove articles that were out of the scope of this review. In case of any doubt, the full text of the article was read. After the evaluation of all abstracts, full-text articles of the selected papers were obtained for further assessment. The reference lists of the selected articles or relevant reviews were searched for any potential articles to be included. Articles were included if they fulfilled all of the following criteria:

1. Original clinical studies of DPC performed on fully formed human permanent teeth with carious exposure.
2. Biodentine was used as a pulp capping material.
3. MTA was included for comparison.
4. At least 6 months of follow-up.
5. The outcome was defined in terms of a clinical and radiographic examination.
6. Published in the English language.

Studies failing to meet any of these criteria were excluded. The two authors independently identified the relevant articles and, in cases of disagreement, a consensus was reached through discussion.

Data extraction

A custom-designed spreadsheet was used independently by two reviewers to extract the following data from included articles: name of the first author, publication year, country, study design, pulpal diagnosis, age of patients, total sample size, tooth type, number and specialty of the operators, isolation

method, use of magnification, method used for bleeding control and disinfection, time needed to control bleeding, placement of the final restoration, follow up, success rate in the MTA group, success rate of Biodentine, and any evidence of tooth discoloration. Any missing information was obtained by contacting the authors.

Assessment of risk bias

The quality of the randomized clinical trials (RCTs) was assessed using the revised Cochrane risk of bias (ROB) tool.²⁶ The following domains were evaluated: randomization process, deviation from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The categories for ROB judgments of randomized trials are of low risk of some concerns and a high risk of bias.

The quality of observational studies was assessed using the ROB for nonrandomized studies interventions tool,²⁷ which covers seven domains: bias due to confounding, bias in the selection of the participants, bias in the classification of interventions, bias due to a deviation from the intended interventions, bias due to missing data, bias in the measurement of outcomes, and bias in the selection of the reported results. The categories for overall ROB judgments in such studies are a low risk, a moderate risk, a serious risk, and a critical risk of bias.

The ROB of each study was evaluated independently by the two reviewers. Any discrepancy in the quality assessment was resolved by discussion.

Data analysis

The statistical analysis was conducted using MedCalc software (version 19.1.1, Ostend, Belgium; <http://www.medcalc.org>). Meta-analysis with the random-effects method was used. The heterogeneity was investigated using the I^2 statistic. The estimated relative risk (RR) and confidence interval (CI) for the outcome measures were computed. A p -value of <0.05 was considered statistically significant. The confidence intervals (CIs) are given at the 95% level. Results were presented in a table and forest plots. Publication bias was not evaluated due to few numbers of included studies.²⁸

Results

Study Selection

The literature search and selection processes are illustrated in Figure 1. After removal of duplicates,

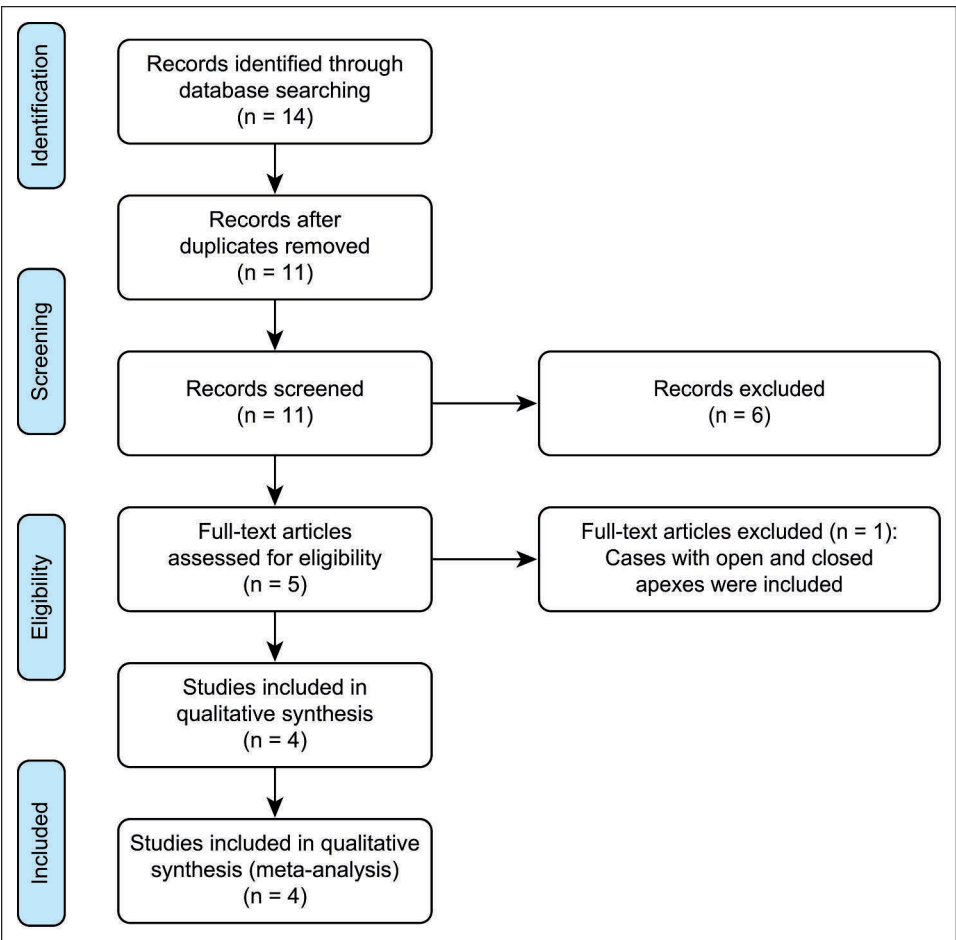


Figure 1. A flow diagram summarizing the systematic review process for article selection according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

the initial search yielded 11 articles that met the initial search criteria. At this stage, the titles and abstracts were screened, and five articles were identified for full-text reviewing. Among these studies, one was excluded,¹⁸ because DPC was performed in molars with open and closed apexes, and the results regarding the success rate in the closed apex group alone was not shown. Finally, four studies were included for this systematic review and meta-analysis.

Characteristics of the Included Studies

The characteristics of the four included clinical studies are presented in Table 2. Three of the selected studies were RCT,¹⁹⁻²¹ and one was a retrospective study.²⁹ The countries of origin of studies were: India,^{19,29} Thailand,²¹ and Jordan.²⁰ The selected studies included a total of 95 participants with an age ranging from 8-51 years. Two studies included molar teeth only,^{19,29} and the other two included anterior and posterior teeth.^{20,21} All teeth had a positive response to sensitivity pulp tests. Linu and others excluded teeth with a

lingering response to pulp tests.²⁹ On the other hand, Parinyaprom and others included cases with early periapical involvement, with a widened PDL space or condensing osteitis.²¹ Furthermore, one study reported the specialty of the operator,²¹ while the other three studies did not report this data.

In all included studies, the treatment procedures were performed under rubber dam isolation with no specific mention of using magnification aids. To achieve hemostasis after pulp exposure, a cotton pellet soaked in sodium hypochlorite (NaOCl) was used. Two studies used a high concentration of NaOCl,^{20, 29} while the other two used a lower concentration.^{19,21} The reported time for bleeding control before the placement of the capping agent varied from 3 to 10 minutes. In one study, the treatment was finalized in a single visit,²¹ while, in the other studies, the final restoration was placed after 1-3 weeks. There was a wide variability in the follow-up periods across the studies, ranging from 6 months to 3 years. However, all studies had a minimum follow-up period of 6 months. Treatment outcomes in all studies were based on clinical

Table 2: Characteristics of the Studies Included in the Review

Study	Country	Design	Age (Years)	Type of Teeth Included	Number of Operators	Operator Specialty	Isolation Method	NaOCl Concentration (%)	Time to Control Bleeding	Final Restoration	Time for Final Restoration	Follow-up (Months)
Hegde and others (2017)	India	RCT	18-40	M	1	NS	RD	3%	NS	Composite	3 week	6
Linu and others (2017)	India	RS	15-30	M	NS	NS	RD	5%	10 minutes	Composite	MTA: 1 w BD: 2 week	6, 12, 18
Parinyaprom and others (2018)	Thailand	RCT	8-18	I, M	8	PG	RD	2.5%	Within 10 minutes	Composite, amalgam, or SSC	Immediate	Range (6-54)
Awawdeh and others (2018)	Jordan	RCT	16-51	I, P, M	NS	NS	RD	5%	3-6 minutes	Composite or amalgam	1 week	6, 12, 18, 24, 36

RCT, randomized clinical trials; RS, retrospective study; I, incisors; P, premolars; M, molars; NS, not specified; PG, Postgraduate residents; RD, rubber dam; NaOCl, sodium hypochlorite; min, minutes; w, week; MTA, mineral trioxide aggregate; BD, Biodentine; SSC, stainless steel crown; NS, not specified.

examinations and radiographic appearance. Additionally, evidence of tooth discoloration was reported in all studies. The study conducted by Parinyaprom and others²¹ was the only study that did not radiographically evaluate the formation of a dentin bridge underneath the capping material.

Results of the Individual Studies

Hegde and others¹⁹ included 24 teeth in their study (MTA=12, Biodentine=12). Three teeth failed within the first 3 weeks (2 with Biodentine and 1 with MTA). At the 6-month follow-up, the success rate for MTA was 91.7%, and the success rate for Biodentine was 83.3%. At the 1-year follow-up, two teeth from the MTA group and two teeth from the Biodentine group showed dentin bridge formation. The authors reported the presence of tooth discoloration in the majority of teeth capped with MTA.

Linu and others²⁹ assessed the outcome of DPC in 26 teeth (MTA=13, Biodentine=13). The success rate in the MTA group was 84.6% (11/13), and it was 92.3% (12/13) in the Biodentine group. None of these cases failed after 1 month. Radiographically, a visible dentin bridge and diffuse calcifications of the pulp chamber were observed for both the groups (69.2% and 7.7% in the MTA group vs. 61.5% and 23.1% in the Biodentine group). Furthermore, 69.2% of cases treated with MTA showed coronal discoloration, whereas no discoloration was noticed in teeth treated with Biodentine.

Parinyaprom and others²¹ compared the outcome of DPC using MTA or Biodentine. Teeth with open and closed apices were included. The authors were contacted to provide additional data for teeth in the closed apices group. The sample size was 28 teeth

(MTA=15, Biodentine=13). No failed cases were reported for all observed follow-up times. Furthermore, discoloration was observed in the MTA group only.

Awawdeh and others²⁰ studied the outcomes of DPC and pulpotomy using MTA and Biodentine. The authors were contacted to provide detailed information about the DPC group. Seventeen teeth were treated (MTA=6, Biodentine=11). At the 1-year follow-up, a tooth from the Biodentine group was excluded, because the coronal restoration was broken, and the pulp was exposed. During the 24-month follow up, all included teeth were considered successful. Radiographically, there was no evidence of a dentin bridge or calcifications. The authors reported the presence of coronal discoloration in all teeth treated with MTA, whereas no discoloration was observed in teeth treated with Biodentine. At the 36-month follow-up, the success rate in the MTA group was 100% (6/6), and it was 88.89% (8/9) in the Biodentine group.

Quality Assessment of the Risk of Bias

Figure 2 shows the quality assessment of the included studies. Among the selected RCTs, only one study presented a low ROB.²⁰ The study by Parinyaprom and others²¹ triggered some concerns, because the clinical evaluation and the decision of the final outcome were performed by unblinded operators. Hegde and others¹⁹ had a high ROB, because they did not report the demographic and baseline clinical characteristics for each group, such as patient age or the type of the treated teeth. Furthermore, no analysis was performed to evaluate the distribution of the partici-

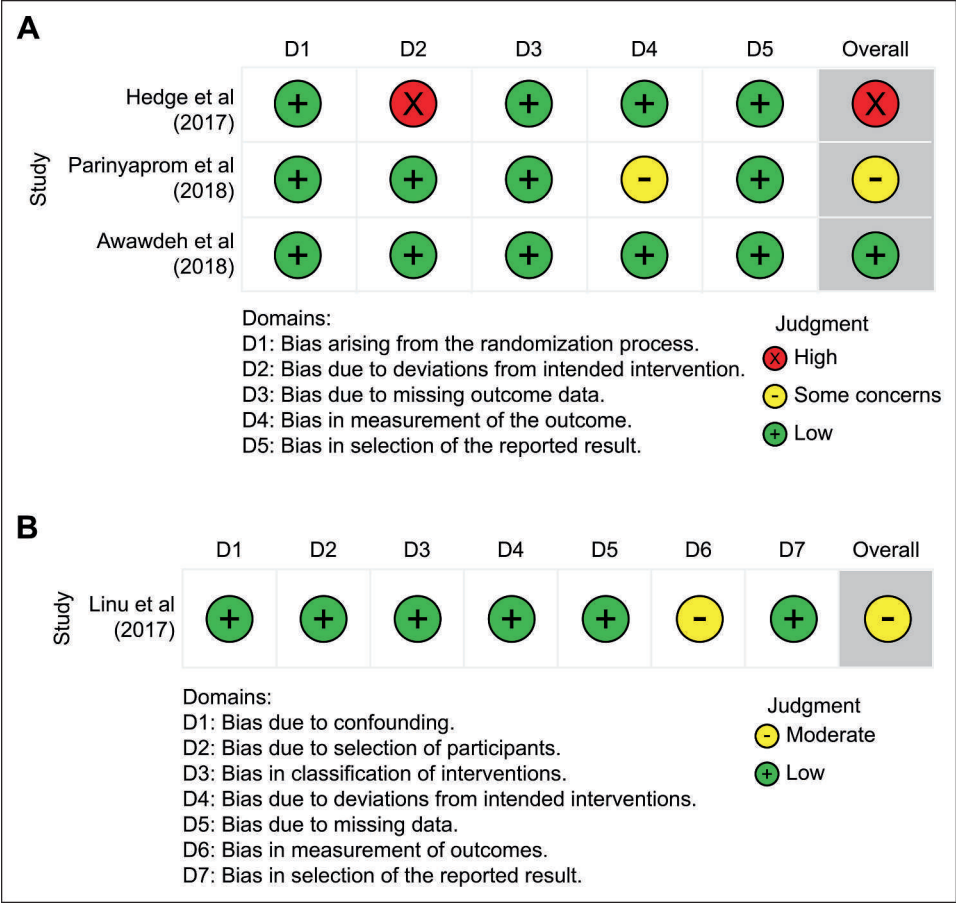


Figure 2. Quality assessment results using (A) the revised Cochrane risk of bias (ROB) tool for randomized clinical trials (RCT) and (B) the ROB for nonrandomized studies of interventions tool for observational studies.

pants between the two groups regarding the baseline variables.

The ROB in nonrandomized studies of interventions tool²⁷ was used to assess the quality of study done by Linu and others,²⁹ which presented a moderate ROB, because no information was provided regarding the operator who assessed the treatment outcome (Figure 2B).

Meta-Analysis

Meta-analysis was performed, considering the success rate of MTA and Biodentine, when used as a DPC agent. As shown in Figure 3, there was no significant difference in the overall success rate between MTA and Biodentine (RR=1.00, CI [0.93-1.07], heterogeneity: $I^2=0.000\%$, $p=1.00$). Due to the limited number of studies that included follow-up period of ≥ 12 months, the subgroup analysis was feasible for 6 months only. The results showed that there was no significant difference in the success of treatment between MTA and Biodentine at 6 months (RR=1.00, CI [0.90-1.11], heterogeneity: $I^2=0.000\%$, $p=0.86$) (Figure 4).

Discussion

Traditionally, carious exposed pulps in mature teeth were managed with complete root canal treatment. However, several recent studies have demonstrated the successful management of such cases using the VPT approach with different calcium silicate-based cements.^{20,21,30} MTA and Biodentine are examples of calcium silicate cements that have similar reported success rates when used as pulp capping agents.^{20,21,30} However, conducting a meta-analysis to determine the differences in the success rates of these materials is important for clinicians to select the best possible pulp capping agent based on the best scientific evidence available. Therefore, this study was focused on comparing the outcome of Biodentine to MTA when used as a DPC agent in mature permanent teeth and carious exposure by using a systematic review with meta-analysis.

The findings of the present review are based on the results from four clinical studies. The overall quality of the included studies was found to have some concerns^{20,21}—to be at a moderate²⁹ or high

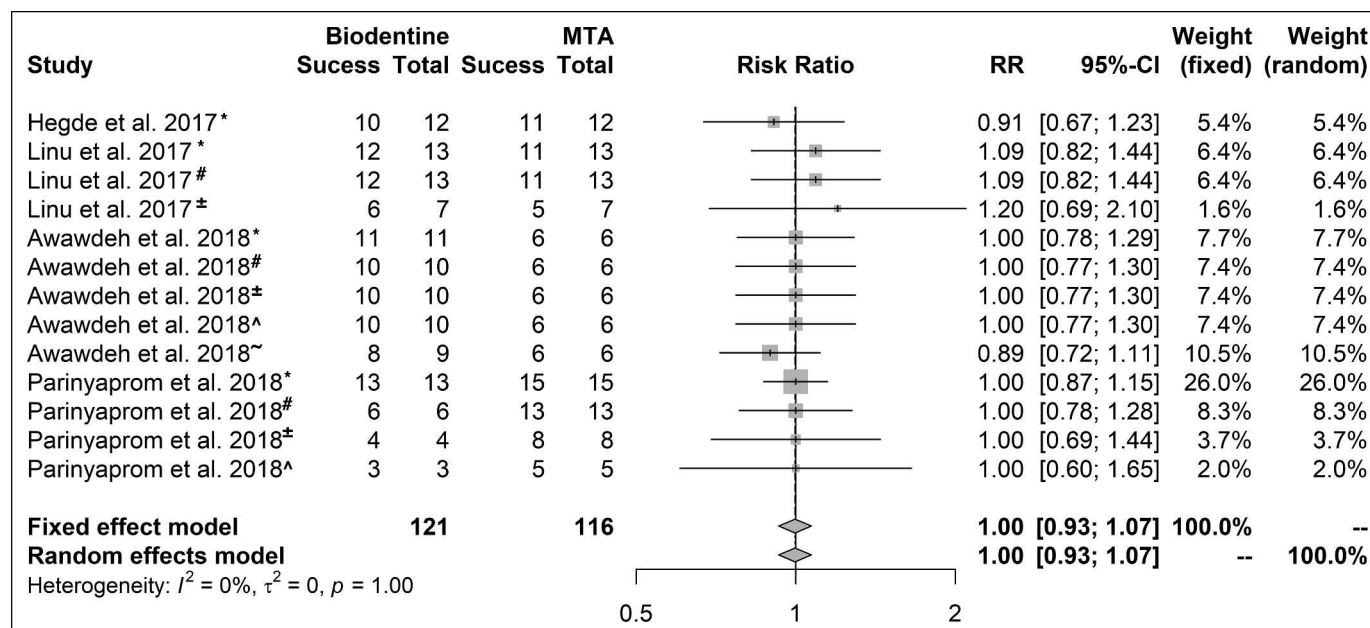


Figure 3. Forest plot comparing the overall outcome of direct pulp capping (DPC) in cariously exposed pulp with Biodentine and mineral trioxide aggregate (MTA). (*) 6, (#), 12, (±) 18, (^) 24, and (~) 36 month.

risk of bias.¹⁹ Only one study mentioned that a blinded assessor evaluated the final outcome.²⁰ Considering the differences between MTA and Biodentine in appearance and handling characteristics, it is not possible to blind the operator. Therefore, performance bias seems to be unavoidable. Furthermore, the study of Hegde and others¹⁹ did not show the baseline clinical characteristics or differences between the two groups. Thus, these results should be interpreted cautiously.

The results of the present systematic review and meta-analysis indicate that there is no difference in the success rate of MTA and Biodentine when used as DPC agents in mature teeth with carious pulp exposure in terms of clinical and radiographic

assessments. The formation of a hard tissue barrier was not included in the present review as an indicator for the treatment success, because it is best evaluated by using histologic sections.³¹ An ideal pulp capping material should be nontoxic, bioactive, and able to bond to the tooth structure.³² MTA and Biodentine have comparable biocompatibility and bioactivity.³³ Furthermore, both materials have similar bond strength to dentin,³⁴ and they formed a mineralized tissue bridge after pulpotomy in an animal experiment.³⁵ In the study by Linu and others,²⁹ diffuse calcifications in the pulp chamber were observed in 7.7% (1/13) and 23.1% (3/13) of cases of MTA and Biodentine, respectively. Total canal obliteration was not observed in any of the

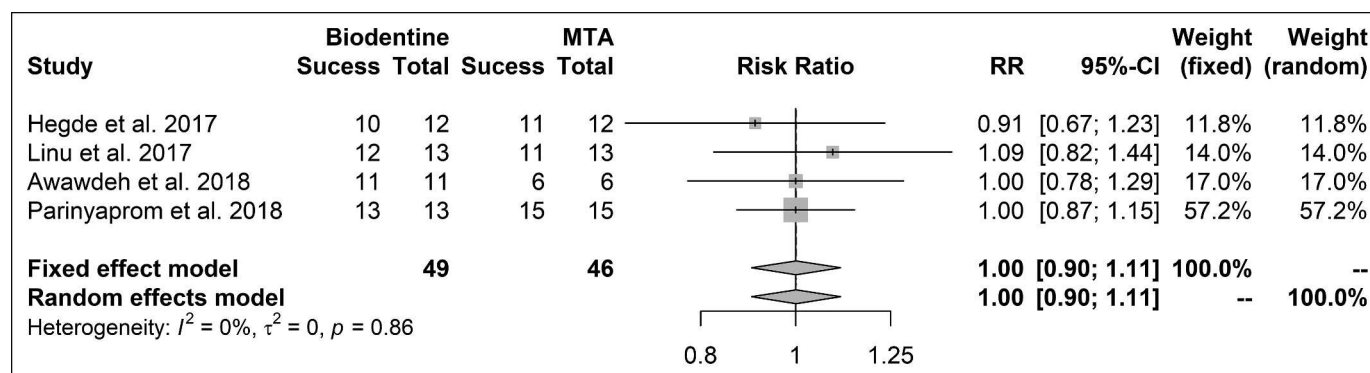


Figure 4. Forest plot comparing the outcome of direct pulp capping (DPC) in cariously exposed pulp with Biodentine and mineral trioxide aggregate (MTA) at 6 month.

included studies. The available literature suggests that the risk of developing an obliteration is higher after complete pulpotomy.³⁶ However, long-term follow-up is required for the evaluation of its incidence after DPC. It is important to note that the findings of this review are consistent with a recent meta-analysis of pooled data from three studies.²⁴ Furthermore, it is consistent with a previous meta-analysis on success rates of DPC in mature and immature teeth.²³

All clinical studies included in this review reported the presence of tooth discoloration in the majority of the cases after the use of MTA, while no discoloration was noted when Biodentine was used.^{19-21,29} This finding is consistent with various *in vitro* studies.^{37,38} The discoloration associated with MTA may be explained by the presence of bismuth oxide as a radiopacifier within the powder. NaOCl was used in all included studies for bleeding control and disinfection before the placement of the capping agent. When bismuth oxide comes into contact with NaOCl, a dark-brown to black discoloration occurs.³⁹ Furthermore, bismuth oxide interacts with dentin collagen and forms a black discoloration.⁴⁰ The lack of bismuth oxide in Biodentine may explain its superior color stability compared to MTA.³⁸ This is of clinical significance, especially for teeth with aesthetic considerations. Considering the other advantages of Biodentine over MTA, including its shorter setting time,⁴¹ better handling characteristics, and lower cost, together with the findings of this systematic review and meta-analysis, Biodentine can be used more confidently for DPC to avoid future patient dissatisfaction.

Factors other than the type of capping material play an important role in the success of the VPT, including control of the pulpal bleeding before placement of the capping agent.⁴² Different methods have been proposed to control pulpal hemorrhage, including the use of saline, NaOCl, or hydrogen peroxide.^{42,43} In all of the included studies, the bleeding was controlled using NaOCl²⁰ or a cotton pellet soaked with NaOCl at concentrations ranging from 2.5% to 5%.^{19,21,29} In addition to its hemorrhage control,⁴³ NaOCl has excellent antimicrobial properties,⁴⁴ which can assist in the disinfection of the perforated area. The adverse effect of microorganisms on pulpal tissue is well known.⁴⁵ Therefore, in these studies, pulp capping procedures were performed under rubber dam isolation to avoid the introduction of microorganisms into the perforated site. In addition to the use of a rubber dam, the final restoration was placed within 3 weeks. The signifi-

cant effect of having a permanent restoration on the favorable outcome of VPT has been reported previously.⁴⁶

The major limitation of this review was that the results of the meta-analysis are limited in terms of the number of cases due to the few available clinical studies that directly compared the outcomes of these two materials in mature permanent teeth. Therefore, additional high-quality RCT with long-term follow-up are required. Another limitation of this review is that the search strategy was restricted to the English language. On the other hand, this review has elements of strength. It has answered a focused clinical question, and the study selection and evaluation process were performed according to standard protocols.

CONCLUSIONS

Within the limitations of this systematic review and meta-analysis, only a limited number of clinical trials comparing MTA and Biodentine as DPC materials is available in the existing literature. Overall, MTA and Biodentine showed comparable clinical and radiographic success rates as DPC agents in mature permanent teeth with carious exposure.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

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Effect Of Calcium Silicate, Sodium Phosphate, and Fluoride on Dentinal Tubule Occlusion and Permeability in Comparison to Desensitizing Toothpaste: An In Vitro Study.

B Rahman • HM El-Damanhoury • S Sheela • HC Ngo

CLINICAL RELEVANCE

Although initially promoted as antiersive or enamel remineralizing toothpaste, calcium silicate, sodium phosphate, and fluoride salts have the ability to reduce dentin permeability more than some of the commercially marketed desensitizing TPs.

SUMMARY

This study compared the ability of a calcium silicate-, sodium phosphate-, and fluoride-based (CSSPF) toothpaste (TP) in promoting dentinal tubule occlusion and reducing dentin permeability with that of other commercially

available antisensitivity TPs. Seventy-eight dentin discs (1.0 ± 0.1 mm thick) were prepared from the midcoronal area and were treated with 37% phosphoric acid for 2 minutes; then they were randomly divided into six groups according to treatments: No treatment [positive control (PC)], entirely covered with nail varnish [negative control (NC)], hydroxyapatite (HAP)-containing TP [Desensin Repair (DES)], NovaMin-based [Sensodyne Repair & Protect (SEN)], CSSPF-based TP [Regenerate Advanced (REG)], sodium monofluorophosphate, potassium citrate, zinc citrate TP [Signal Sensitive Expert (SIG)]. Dentin permeability was tested by the dye percolation method (DP%). Scanning electron microscope (SEM) micromorphological and energy dispersive X-ray elemental analysis (EDX) of the dentin surfaces were done following each treatment. Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test at a 95% confidence level ($\alpha=0.05$). All the tested groups showed higher DP% than NC

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and lower percolation than the PC ($p < 0.05$). REG and SIG were statistically comparable, and showed significantly lower DP% ($p < 0.05$) than SEN and DES. None of the TPs tested was able to obliterate the lumen of the dentinal tubules (DT) completely. REG exhibited the highest weight percentage of calcium deposition, followed by SEN. Compared to the tested desensitizing TPs, CSSPF-based TPs demonstrated equal or less dentin permeability and better DT occlusion.

INTRODUCTION

Dentin hypersensitivity (DH) has been described as the short sharp pain caused by exposed dentin that cannot be ascribed to any other dental disease.^{1,2} Brännström and others proposed the hydrodynamic theory to explain DH as the fluid movement in open dentinal tubules (DT) when they are exposed to thermal, evaporative, tactile, osmotic, or chemical stimuli, which activate mechanoreceptors that cause pain and discomfort.^{3,4} Gingival recession or loss of enamel due to abrasion, erosion, or abfraction may cause the exposure of DT.⁵ Pain arising from exposed DT may hamper the patient's maintenance of good oral hygiene.⁶ It was noticed that the increased number of exposed DT were closely correlated to the increased sensitivity.⁷ Two methods were proposed to desensitize dentin: 1) Block out or modulate the response of intradental nerves to fluid movements or 2) mechanically occlude the exposed DT to reduce dentin permeability and prevent fluid movement.⁸

Potassium ions present in some types of toothpastes (TPs) in the form of 5% potassium nitrate may reduce tooth sensitivity by decreasing the excitability of A-fibers around the odontoblasts.⁹ However, dentinal tubule occlusion is the most common therapeutic method.⁸ Most of the home-use desensitizing products belong to the second group and include a wide range of active elements. One of these active elements is Bioglass. It was initially developed to stimulate new bone formation. It was also shown by scanning electron microscopy (SEM) that it occludes DT when applied to the exposed dentin by forming a hydroxycarbonate apatite (HCA) layer over the tubules.¹⁰ NovaMin, which is based on the original 45S5 Bioglass, is the active ingredient in Sensodyne Repair & Protect dentifrice; a number of clinical and laboratory studies showed the ability of this product to block DT and relieve sensitivity.¹¹⁻¹⁸

The combination of arginine and calcium carbonate demonstrated the ability to occlude DT by the precipitation of calcium phosphates. However, these

precipitates are soluble and unstable under acid challenge.¹⁹ Clinical studies have demonstrated a significant reduction in DH after the use of arginine and calcium carbonate TPs immediately and up to 8 weeks after treatment.^{20,21} Another approach to treat DH is the use of stannous fluoride, which is characterized by water and acid-resistant precipitate, which demonstrated effective treatment of DH in many randomized controlled clinical trials.^{22,23}

Another novel approach is the use of nanoparticles as desensitizing agents.^{24, 25} Nanoparticles may easily penetrate DT and block fluid movement due to their small size. A recently introduced formulation containing calcium silicate, sodium phosphate, and fluoride (CSSPF), promoted initially as a remineralizing or antierosive TP for the repair of demineralized enamel, was found to deposit calcium silicate particles onto the enamel surface and nucleate HAP formation.²⁶ This mechanism of action can be very appealing to use for the obliteration of the DT and the treatment of DH. There are few reports in the literature on the potential repair of dental enamel by CSSPF formulations^{27,28}; however, no information is available on their effect on dentin lesions. Therefore, the objectives of this study were to compare the ability of CSSPF formulations to promote dentinal tubule occlusion and reduce dentin permeability, with that of other commercially available antisensitivity TPs. The null hypothesis was that there is no difference between CSSPF-based TP and the anti-sensitivity TPs tested in their ability to occlude the DT and reduce dentin permeability.

METHODS AND MATERIALS

Preparation of Dentin Samples

Seventy-eight caries-free freshly extracted human third molars were obtained from the oral surgery clinic after the patient's informed consent was obtained. The teeth were cleaned from soft tissue remnants with an ultrasonic scaler and stored in 0.1% thymol solution for one month before use. Seventy-eight dentin discs with a thickness of 1.0 ± 0.1 mm were prepared from the midcoronal area by sectioning over the cemento-enamel junction (CEJ) using a low-speed, water-cooled diamond saw (Iso-Met 1000, Buehler Ltd, Lake Bluff, IL, USA). The dentin discs were inspected under the stereomicroscope to exclude the presence of pulp horn exposures or remnants of enamel. The disc samples were collected and polished with 600-grit silicon carbide paper to create a standardized smear layer, and then the samples were trimmed using a diamond finishing bur to adjust their diameter to 8.5 ± 0.1 mm.

Table 1: *Materials Tested in This Study*

Product	Abbreviation	Manufacturer	Active ingredient	LOT
Desensin Repair	DES	Dentaid, Spain	Nano hydroxyapatite (HAP), potassium nitrate	K2011
Sensodyne Repair & Protect	SEN	Glaxo Smith Kline, Weybridge, Surrey, UK	Glycerin, silica, calcium sodium phosphosilicate (Novamin), sodium monofluorophosphate 1.08% w/w (1450 ppm fluoride)	077G L1
Regenerate Advanced	REG	Unilever, UK	Glycerin, calcium silicate, hydrated silica, trisodium phosphate, sodium phosphate, sodium monofluorophosphate (1450 ppm fluoride)	L4274CCC
Signal Sensitive Expert	SIG	Unilever, UK	Sodium monofluorophosphate (1450 ppm fluoride), potassium citrate, zinc citrate, HAP	B21

Dentin Permeability (Dye Percolation Test)

A circular paper sticker with 5.5-mm diameter was placed on each prepared dentin disc's surface, and nail varnish was applied to the exposed dentin around the sticker. The nail varnish was left for 5 minutes to dry, then the sticker was removed, and the upper and lower surfaces of the dentin discs were treated with 37% phosphoric acid gel for 2 minutes to remove the surface smear layer and attain patent tubules of dentin. The specimens were rinsed with distilled water and maintained wet to evaluate the maximum permeability. The dentin discs were randomly divided into six groups, 13 samples each, and received one of the following treatments:

- Group 1 [positive control (PC)]: No treatment
- Group 2 [negative control (NC)]: Samples were entirely covered with nail varnish.
- Group 3 (DES): Brushed with potassium nitrate-, HAP nanoparticles-containing TP.
- Group 4 (SEN): Brushed with NovaMin bioactive glass (BG)-containing TP.
- Group 5 (REG): Brushed with a calcium silicate-, sodium phosphate-, and fluoride- containing TP.
- Group 6 (SIG): Brushed with sodium monofluorophosphate-, potassium citrate-, zinc citrate-containing TP.

The composition of the materials tested are listed in Table 1. Each TP (a slurry of 1 g of TP and water in 1:2 ratio) was applied for 2 minutes two times a day for 7 days, using a soft brush and minimal pressure; the dentin surface was rinsed with distilled water after every application and kept immersed between the applications in artificial saliva (composition: 0.4 g NaCl, 0.4 g KCl, 0.795 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.78 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.005 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 1 g urea, and 1000 mL distilled water).

Sixty plastic vials (Eppendorf tube, Eppendorf AG, Hamburg, Germany) were cut at 5 mm from the vial's tip, using a cutter, and the inner surfaces of

the vials were sandblasted with 25- μm aluminum oxide particles. The vials were cleaned with distilled water and dried, and a resin bonding agent (Scotch-bond Universal, 3M Oral Care, St. Paul, MN, USA) was applied at the inner surface of the vials at a level of 5 mm from the vial's opening. Ten dentin discs from each group ($n=10$) were randomly selected and placed in the vials at the level of 5 mm, and flowable composite (Filtek Z350 XT, 3M Oral Care, St. Paul, MN, USA) was used to seal the interface between the side of the disc and the inner wall of the vials. The composite was light-cured using an LED light-curing unit (Elipar, 3M Oral Care, St. Paul, MN, USA). The dentin disc divided the vial into an upper compartment and a lower compartment (Figure 1). The vials were numbered, the lower compartments were filled with distilled water, and the caps were closed. The lower compartments were checked for the presence of any air bubbles in the water, and, if a bubble was detected, injection of water through the vial's wall using a fine needle syringe occurred, and the puncture site was closed with paraffin wax.

A two-toned dye (Plaquesearch, Oral Dent Ltd, UK) was used to test dentin permeability. Different concentrations of the dye were made, considering the undiluted as 100%, and serial dilutions were made from 100%-3.125% (Figure 1). The absorbance of the different concentrations of the dye was read in a multimode microplate reader (Varioskan Flash, Thermo Fisher Scientific, Vantaa, Finland) at a wavelength of 630 nm and plotted to generate a calibration curve. The calibration curve was optimized to get a straight line by including the dilutions from 0.39% to 3.125% only. 100 μL of 3.12% dye was added to the upper compartment of the vial, on top of 10 samples from each group ($n=10$), and kept undisturbed for 24 hours. The readings were taken in the microplate reader, and, using the calibration curve, the equivalent dye percentage of the readings was recorded.

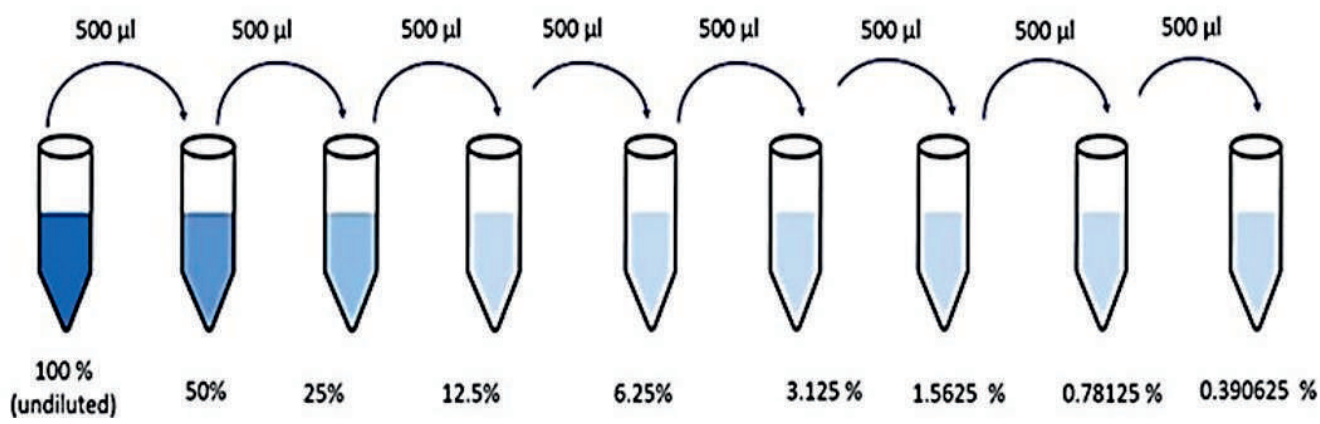
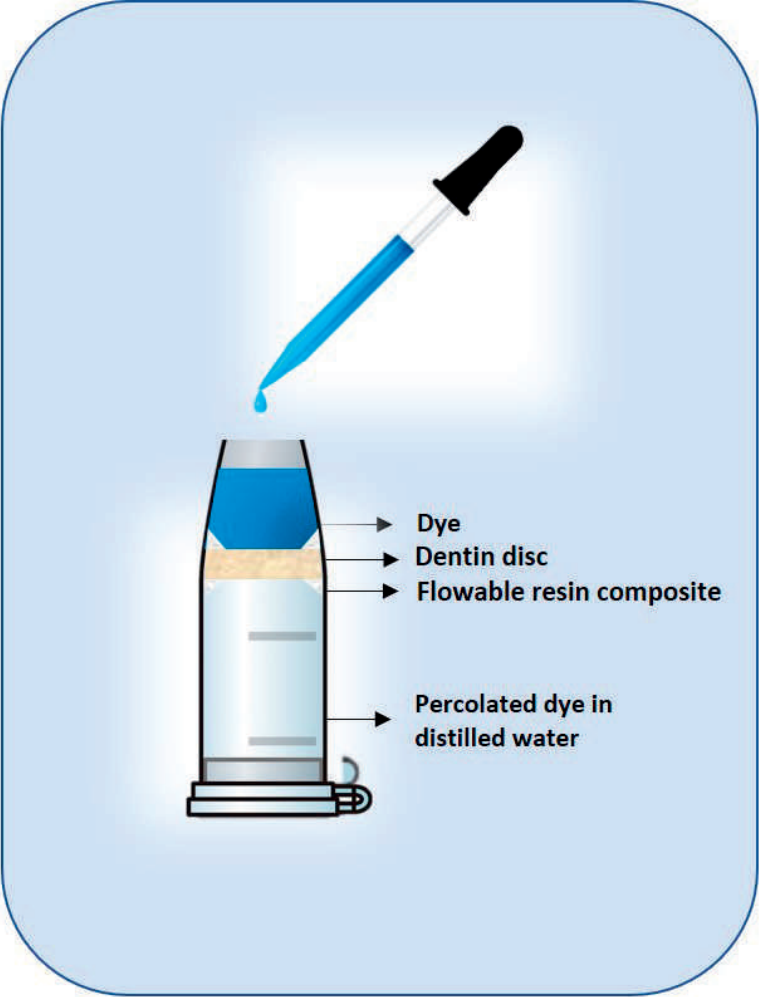


Figure 1. Schematic representation showing the sample set-up for the dye percolation (DC) test and the different dilutions of dye for making the calibration curve (considering the undiluted solution as 100%).

Table 2: Dye Percolation (DC) (Mean%± Standard Deviation) and Energy Dispersive X-ray Elemental (EDX) Analysis of Major Components of Element Surface Composition (wt%) of the Dentin Surface after Treatment with the Tested Materials

Material	Percentage Dye Percolation (DC%)	Elemental Surface Composition (wt%)				
		Ca	P	Si	C	O
NC	0.003 ± 0.002 a	0.0	0.0	0.7	26.5	17.4
SIG	0.054 ± 0.028 b	9.85	4.6	0.9	43.6	27.5
REG	0.064 ± 0.020 b	21.6	10.1	0.9	27.2	39.2
DES	0.108 ± 0.025 c	12.9	8.4	1.8	29.5	39.9
SEN	0.126 ± 0.040 c	19.1	8.6	1.8	30.1	38.4
PC	0.195 ± 0.050 d	8.0	3.6	1.3	59.9	20.5

Mean values with different lowercase letters are statistically significantly different ($p < 0.05$).

Micromorphological and Energy Dispersive X-Ray Analysis (SEM-EDX)

The three remaining dentin discs samples from each group ($n=3$) were dehydrated in a desiccator containing dehydrated silica gel at room temperature for 24 hours. Samples were sputter-coated with 100 Å gold-palladium (EMS 7620 Mini Sputter Coater, Hatfield, PA, USA) to create a conductive specimen surface and reduce electron charging, which may reduce the quality of the image. The treated surfaces of the samples were analyzed, and one sample from each group was fractured using a #15 scalpel blade to reveal the cross-section of the samples and study the penetration of the TP into the dentinal tubules. Observations were performed under different magnifications up to 5000×, using a scanning electron microscope (SEM) (VEGA3 XM—TESCAN, Kohoutovice, Czech Republic) operating at 10-20kV acceleration voltage and 10-27 mm working distance.

Elemental analysis of the samples from each group was done using a SEM-coupled energy dispersive X-ray microanalyzer (EDX - TESCAN, Kohoutovice, Czech Republic), operating at a take-off angle of 35° with map mode. The average main element percentage weight content for each TP (silicon, fluoride, calcium, and phosphorus) was calculated.

Statistical Analysis

The power analysis of the study was performed after a pilot study to determine adequate sample size. One-way analysis of variance (ANOVA) and Tukey post hoc tests were used to detect pairwise differences among experimental groups. A 95% confidence level was applied for all the statistical tests, and the power of the analysis with $\alpha=0.05$ ($b=0.10$, $f=0.40$) was 0.90. The statistical analysis was performed by the IBM SPSS Statistics software (SPSS version 20.0, SPSS Inc, Chicago, IL, USA).

RESULTS

Dentin Permeability (Dye Percolation Test)

The dentin permeability results, expressed as dye percolation percentage (DP%), are shown in Table 2. The one-way ANOVA test revealed that the type of TP significantly affected dentin permeability ($p < 0.0001$). Compared to the NC group, all the tested groups showed statistically higher DP% ($p < 0.05$). Meanwhile, all the tested materials exhibited significantly less percolation than the PC group (0.195 ± 0.05). There were no statistically significant differences ($p > 0.05$) in DC results among REG and SIG (0.064 ± 0.02 and 0.054 ± 0.028 , respectively), which have shown significantly less DC ($p < 0.05$) than SEN (0.126 ± 0.04), and DES (0.108 ± 0.025).

Micromorphological and Energy Dispersive X-Ray Analysis (SEM-EDX)

The SEM microphotographs of aerial views of sample surfaces and cross-sectional views of the fractured samples for each group are illustrated in Figure 2. The PC samples showed open DT with a complete absence of smear layer and plugs, while the analysis of the NC group revealed a 10- μ m-thick nail varnish layer on the surface with up to 15- μ m-long intact smear plugs filling the DT. In comparison to the NC group, none of the TPs tested in this study was able to obliterate the lumen of the DT completely. Deposition of crystal-like and granular structures on the dentin surface and in the lumen of the tubules brushed with SEN, SIG, REG, and DES resulted in a reduction of the tubule lumen diameters (arrow) and complete obliteration of some tubule openings. A longitudinal view of the fractured samples revealed poor penetration of the TPs into the DT, except for REG and SIG, which showed deeper penetration into the DT up to 15 μ m.

EDX micrographs are presented in Figure 2, and the analysis of major components of the surface

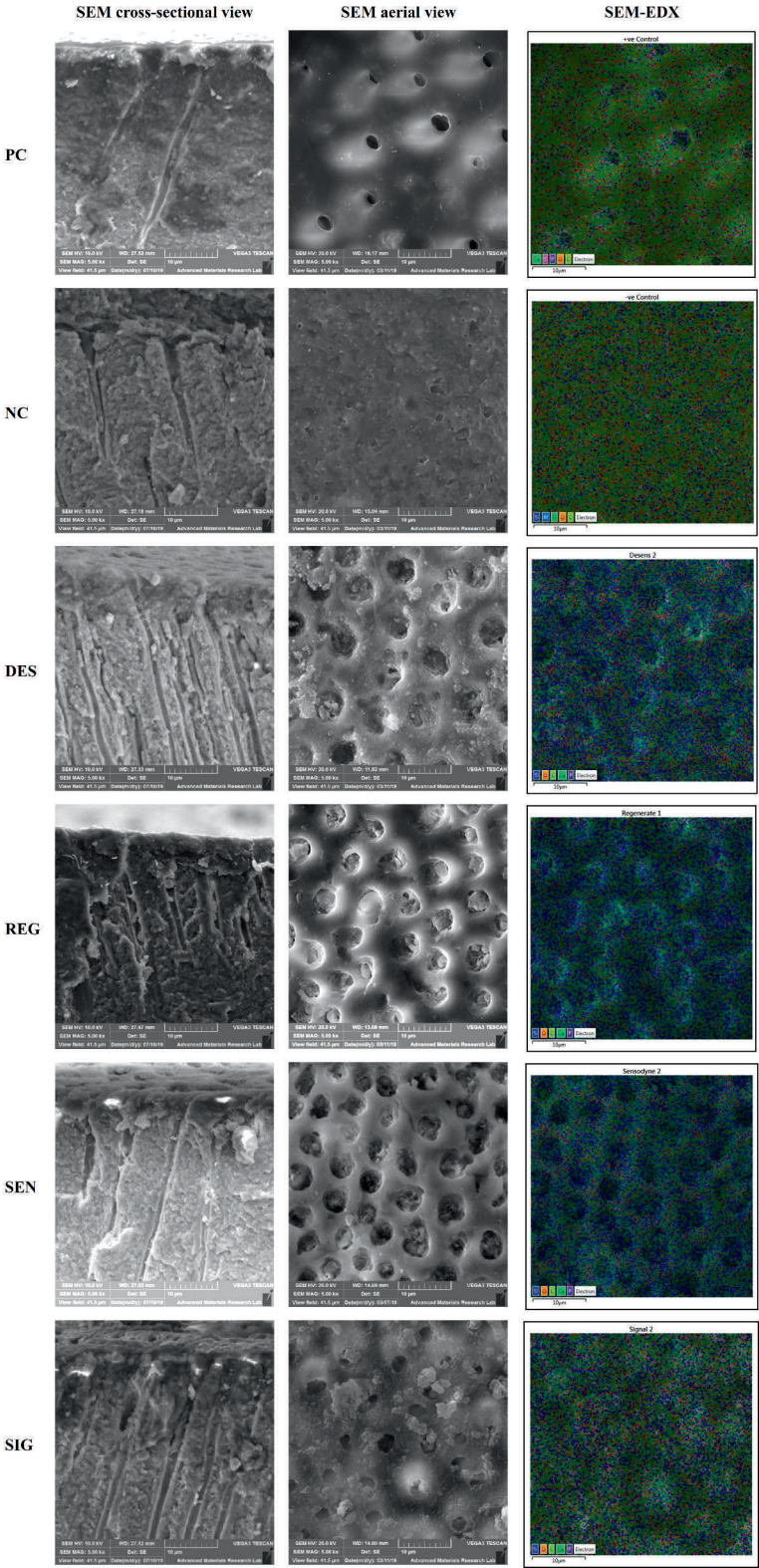


Figure 2. Scanning electron microscope (SEM) (cross-sectional view and aerial view) and energy dispersive X-ray elemental analysis (EDX) micrograph (5.0 kx magnification) of dentin disc, after 37% phosphoric acid-etched dentin surface (Positive Control, PC), fully covered with nail varnish (Negative Control, NC), and after treatment with Desensin Repair (DES), Regenerate Advanced (REG), Sensodyne Repair & Protect (SEN), and Signal Sensitive Expert (SIG) toothpastes.

elemental composition of the dentin disc surfaces is shown in Table 2. Some elements, including phosphorus (P), silicon, (Si), chlorine (Cl), sodium (Na), and magnesium (Mg), were detected, but the most predominant element was calcium (Ca), with REG exhibiting the highest weight percentage of Ca (21.6 %) followed by SEN (19.1 %).

DISCUSSION

Fluid conductance in the DT is the main contributing factor to DH; therefore, one of the fundamental approaches to manage DH aims at occluding the exposed DT and consequently preventing the movement of fluid in the tubule, and thereby reducing the pain and discomfort associated with DH. The current study investigated the efficacy of an antiersive calcium silicate/sodium phosphate salts/fluoride TP [Regenerate Advanced (REG)] in comparison to three different anti-sensitivity TP preparations in occluding the DT.

There are various methods available in the literature to test the dentin permeability.^{29,30} Pashley and others described one of the most widely used methods in which a fluid filtration system and split-chamber unit were used to measure the dentin permeability.³¹ In this method, the movement of an air bubble is measured, and the linear displacement is translated into a dentin permeability value. Several other *in vitro* studies utilized SEM topographical examination to measure the percentage of dentinal tubule occlusion.³² Knight and others described testing dentin permeability through the bacterial migration model, in which a chemostat system provides a constant supply of *Streptococcus mutants* that passes through the dentin discs. The permeability is assessed through optical density measurement and is proportional to the bacterial growth in the nutrient broth.³³

In our study, we have used a DC method, which calculated the percentage dentin filtration of a known concentration of a specific dye through the DT. The dye concentration is measured utilizing a microplate reader at a particular wavelength and compared against a calibration curve to obtain the percentage of dye filtration through the dentin disc. When compared to Pashley and Knight methods, the DC method is easier to set up, reasonably reliable, reproducible, and more cost-effective. The results of the current study showed that all four types of TPs tested were found to significantly decrease the dentin permeability when compared to the PC group. Therefore, the null hypothesis should be rejected. Out of the four TPs, REG and SIG exhibited

significantly less DC ($p < 0.05$) than the two other materials. Most of the studies reported on calcium silicate/sodium phosphate/fluoride (REG) were on the antiersive effect or enamel remineralization capacity of this TP.^{26,27}

The only study that tested the REG efficacy on dentin permeability is a recent study by João-Souza and others, where REG showed a significant decrease in dentin permeability when compared to all desensitizing TPs (arginine, calcium sodium phosphosilicate, strontium acetate, and stannous fluoride) tested.³⁴ The results of our study are in agreement with the reported findings of the João-Souza study. We found that REG effectively reduces dentin permeability, similar to the desensitizing TPs or even better.

The ability of REG to reduce the dentin permeability is mainly attributed to the effective dentinal tubule occlusion, as seen in the SEM topographical analysis (Figure 2), and the unique composition of multiple salts that enhance the deposition of complex crystals in the tubules and possible chemical interaction with the dentinal minerals. Several studies have described the mode of action of REG on enamel remineralization, which is based on the dual-phase gel system containing calcium silicate and phosphate, with 1450 ppm fluoride ion from the sodium fluoride and sodium monofluorophosphate—a system that favors ion exchange, and the nucleation and growth of HAP crystals on enamel.^{26,35} We postulate that a similar mode of action occurs when brushing REG on dentin. The pH for unbuffered CaSi-water rises to a pH of 9.1. This alkaline environment in the presence of sodium phosphate salts enhances the deposition of calcium silicate in the DT. This postulation is confirmed by the EDX elemental analysis (Figure 2), which showed that among all the tested TPs, the highest calcium contents were reached with REG.

Although SEN has a similar composition to that of REG (calcium sodium phosphosilicate, sodium monofluorophosphate, and 1450-ppm fluoride), it exhibited significantly higher DC. Meanwhile, SIG, which has potassium citrate, zinc citrate, and HAP as active components, has shown comparable results to REG, significantly higher than SEN and DES ($p < 0.05$). Jena and others reported similar results when they compared a TP containing potassium citrate and zinc citrate (Pepsodent Pro-sensitive Relief and Repair), with Sensodyne Repair & Protect showing significantly higher dentinal tubule occlusion on the former than the latter.³⁶ Farooq and others demonstrated superior dentinal tubule occlu-

sion by HAP-containing TPs and BG-containing TPs compared to acid-etched control and fluoride TPs.³⁷ The findings of the current study support the results of these reports, as DES (HAP-containing TP) and SEN (BG-based TP) were found to reduce the DC in comparison to the PC significantly. Yuan and others have shown a significant improvement in dentinal tubule occlusion by adding HAP to ordinary TP in their study.³⁸ A novel BG–ceramic, which contains sodium-calcium phosphate and HAP, was compared to commercial BG-containing TP (NovaMin) regarding their dentin permeability reduction capability; no significant difference was observed, as both of them were found to reduce dentin permeability and occlude DT.³⁹

One limitation of this study was the application of the desensitizing TPs for a limited time of 7 days only, and that the specimens were stored in artificial saliva between applications. Longer application of the TPs or subjecting the samples to acidic challenges may result in different outcomes. Supporting the findings of this research with more clinical studies is recommended for future investigations.

CONCLUSIONS

Although all the tested TPs were able to reduce the fluid percolation through the dentin and occlude the DT to various extents, the antierosive calcium silicate-, sodium phosphate-, fluoride-containing TP showed significantly better results than some of the commercially marketed desensitizing TP. The superior performance of this TP may be attributed to the combination of different salts in a highly alkaline media that promote remineralization and crystal formation, and subsequently tubule occlusion.

Conflicts of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this manuscript.

Regulatory Statement

This protocol was approved by the University Research Ethics Committee (approval number: REC-17-11-19-01) and was performed in conformity with the University's guidelines for handling biological tissues.

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Restoration of Severely Damaged Endodontically Treated Premolars: Influence of the Ferrule Effect on Marginal Integrity and Fracture Load of Resin Nano-ceramic CAD-CAM Endocrowns

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A Feilzer • CM Saratti • I Krejci

Clinical Significance

Endocrowns are a valuable alternative to classical post-and-core crowns for the restoration of severely damaged premolars. The adjunction of a ferrule to the endocrown design does not seem to have an impact on its marginal integrity and load-bearing capacity.

SUMMARY

Objectives: To explore the ferrule effect on Resin Nano-Ceramic (RNC) premolar endocrown marginal integrity and fracture resistance.

Methods: Thirty-six root–canal-treated premolars were cut 2 mm above the cemento-enamel junction

(CEJ). They were restored with standardized RNC computer-aided design/computer-aided manufacturing (CAD/CAM) restorations (Lava Ultimate, 3M Oral Care, St. Paul, MN, USA) and divided into three groups (n=12): endocrowns with a 3-mm endo-core (Group 1), endocrowns with a 3-mm endo-core and a 2-mm ferrule (Group 2), and

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conventional post-and-core crowns with a 2-mm ferrule (Group 3). All specimens were submitted to thermo-mechanical cycling loading (TMCL) (1.7 Hz, 49 N, 600 000 cycles, 1500 thermocycles). Margins were analyzed before and after the TMCL. In the second part of the experiment, the surviving specimens were submitted to unidirectional monotonic loading until fracture. Fragments were then analyzed using scanning electron microscopy (SEM) and the fracture mode was established.

Results: Endocrowns of Groups 1 and 2 performed better than conventional crowns (Group 3) in terms of marginal continuity. Differences in fracture load values within all groups were not statistically significant. Most of the specimens fractured in a non-repairable way.

Conclusions: The present test failed to provide evidence of any kind of difference between endocrowns with and without a ferrule in terms of load-bearing capacity and marginal integrity after fatigue. Further studies are needed to confirm the role of the ferrule in adhesive restorations of endodontically treated teeth.

INTRODUCTION

This research addresses the restoration of severely damaged endodontically treated premolars. The most common technique used for the reconstruction of extremely damaged teeth consists of fabricating a porcelain fused to metal (PFM) crown over an internal core which is built around a cemented radicular post. Today, restorative strategies based on adhesive techniques, such as direct composite resins and indirect CAD-CAM workpieces, are reliable alternatives to this classical approach.

Within the category of indirect restorations, endocrowns are becoming more and more popular in clinics. These are overlays made of monolithic ceramic or composite resin that restore the coronal portion of a root–canal-treated tooth by means of an extension inside the former pulp chamber called the endo-core. This extension provides stability and increases the adhesive surface, improving retention of the coronal part. Endocrowns present several advantages over classical crowns. For one thing, the absence of a deep anchoring in the root allows the preservation of more residual dentinal structure and reduces complications associated with long post placement such as root perforation or root fracture.^{1,2} Moreover, the fabrication of a core inside an endocrown is not needed. This simplification of the clinical procedure is associated with

reduced costs, especially for CAD/CAM restorations made chair-side.³ In addition, endocrowns may present an easier obstacle when performing endodontic retreatments, as the restoration extends inside the root for a few millimeters, and no metal is included in the restoration. Nevertheless, concerns may arise when this kind of restoration breaks. The absence of a metallic substructure could leave subjacent tissues exposed to catastrophic vertical fracture.^{4,5}

It is recognized that endodontically treated teeth (ETT) are more prone to fracture than vital ones.⁶ The fracture resistance of ETT depends on many factors, such as the loading context, the type of restoration, and the quality and quantity of residual tissues.^{7,8,9} The use of a ferrule as a reinforcing element in residual tissues is a widely debated issue.^{10,11} The ferrule's mechanism of action relies on the protective role of the cervical part of the prosthetic crown that surrounds the residual tooth structure and acts as a ring that avoids the deflection of residual tissues and strengthens the tooth. Moreover, this strip of tissue improves the contact between the prosthetic crown and the rest of the root, thus enhancing its retention. According to classical prosthetic principles, the ferrule should be a minimum of 1 mm to 2 mm high, have parallel axial walls, encircle the tooth and end on sound tooth structure. In the scientific literature, many studies, the majority of which refer to conventional crown restorations, confirm the positive effect of a ferrule when restoring ETT.^{4,12,13,14,15,16,17}

The favorable influence of a ferrule-integrating cavity beneath large, adhesive indirect restorations such as endocrowns for the restoration of devitalized teeth still needs to be proved. Few *in vitro* studies exist on this topic. Einhorn and others⁴ showed that the addition of a ferrule into the cavity configuration of lithium disilicate molar endocrowns increased their fracture strength, with no difference in load-bearing resistance between preparations showing a 1 mm- or 2 mm-high ferrule in dentin. Similar findings were found by Taha and others¹⁸ for polymer-infiltrated ceramic-network (PICN) molar endocrowns. Rocca and others¹⁹ showed that when compared to classical post-and-core crown restorations, premolar endocrowns made of lithium disilicate-reinforced ceramic with butt margins (no ferrule) performed as well as conventional crowns (with ferrule) in terms of marginal integrity and resistance after fatigue.

The aim of this study was to investigate the influence of the “ferrule effect” on marginal adaptation, fracture resistance, and mode of fracture of RNC CAD/CAM premolar endocrowns. The integration of a ferrule into the endocrown cavity design was tested and compared

to conventional crowns with post and core. Considering both the marginal adaptation (before and after cyclic stress) and load-bearing capacity of the restorations, it was hypothesized that a) the presence of the ferrule improves endocrown performance, and b) endocrowns perform better than crowns.

METHODS AND MATERIALS

Thirty-six recently extracted human upper premolars of approximately the same size were used in this study. The inclusion criteria were the absence of carious lesions and visible crack lines in the root and a complete root formation. Bucco-palatal and mesio-distal dimensions as well as the root length of each tooth were measured using a digital caliper. The teeth were then stored in a sodium azide solution (0.2%) at 4°C until the experiment onset.

Endodontic Procedure

All teeth were endodontically treated. The pulp chamber of each specimen was opened with diamond burs following a standardized procedure. Working length was determined visually by passing a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal until its tip was visible from the apical foramen. The root canal preparation was performed using nickel-titanium (NiTi) rotary instruments (ProTaper U, Dentsply) until the F3 instrument (ISO 30) in a low-speed handpiece (X-Smart, Dentsply) under intermittent rinsing with 4.2% sodium hypochlorite. All instrumentation was performed according to the manufacturer's instructions. A set of ProTaper U was replaced after the preparation of four canals. A resin-based endodontic sealer (AH Plus, Dentsply-DeTrey, Konstanz, Germany) and warm gutta-percha were inserted into the root with a vertical condensation technique (System B and Elements Obturation Unit gutta-percha extruder, Kerr Dental, Orange, CA, USA). Teeth were stored for 24 h in distilled water at 37°C before cavity preparation.

Cavity Preparation

Each specimen was placed on a metallic holder (Baltec, Balzer, Liechtenstein) and fixed in a vertical position by means of light-curing composite; the root base was then embedded with self-curing acrylic resin up to 1 mm below the cemento-enamel junction (CEJ) to obtain full stabilization of the teeth. The crown of each tooth was horizontally sectioned with a diamond disk (Kerr Dental, Brea, California) 2 mm occlusal to the CEJ. The teeth were then randomly assigned to three groups (n=12). In Group 1, coronal tissues were horizontally cut at the CEJ, and gutta-percha was removed from the

pulp chamber until 3 mm below the CEJ. For Group 2 and 3 specimens, a peripheral chamfer (1 mm wide, 2 mm high) was integrated to create a ferrule effect, and gutta-percha was removed as in Group 1. In all prepared teeth, mesio-distal and bucco-palatal dimensions were measured with a digital caliper $3.3 \text{ mm} \pm 0.2 \text{ mm}$ and $6.5 \text{ mm} \pm 0.2 \text{ mm}$ respectively. All procedures were performed using coarse diamond-coated burs (Cerinlay No. 3080.018 FG, Intensiv, Viganello, Switzerland) and finished with fine-grained burs of the same shape (Cerinlay No. 3025.018 FG, Intensiv) under abundant water spray cooling. All cavity dentin surfaces were sealed with a self-etch adhesive system (Scotchbond Universal Adhesive, 3M Oral Care) according to the manufacturer's recommendations. The bonding resin was polymerized for 20 seconds with a second-generation LED high-power device (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) which delivered a power density of 1200 mW/cm^2 . After the immediate dentin-sealing procedure, for Groups 1 and 2, digital impressions were made (Cerec Omnicam, Dentsply Sirona, York, PA, USA). Then the cavities were coated with a water-based glycerine gel (Airblock, Dentsply), and teeth were provisionally restored with a soft light-curing resin (Fermit N, Ivoclar Vivadent) and kept in saline solution for 7 days at 32°C. For Group 3, a pre-calibrated glass-fiber post (RelyX Fiber Post, 3M Oral Care) was inserted into the palatal canal for a length of 5 mm and luted with universal dual-curing resin cement (RelyX Ultimate, 3M Oral Care) following the manufacturer's recommendations. Then a 3.5-mm-high core was fabricated with a restorative nano-hybrid composite resin (Filtek Supreme XTE Universal Restorative, A2 shade, 3M Oral Care); digital impressions were made (Cerec Omnicam, Dentsply) and teeth were provisionally restored as for Groups 1 and 2.

Fabrication of the Restorations

The prepared premolars were divided into three groups and restored with RNC CAD/CAM material (Lava Ultimate, 3M Oral Care) as follows (n=12):

- Group 1: CAD/CAM RNC endocrowns with a 3-mm integrated endo-core.
- Group 2: CAD/CAM RNC endocrowns with a 2-mm integrated ferrule and 3-mm integrated endo-core.
- Group 3: CAD-CAM RNC post-and-core crowns with a 2-mm integrated ferrule.

A schematic representation of the experimental design of this study is shown in Figure 1.

All restorations were created with nearly identical

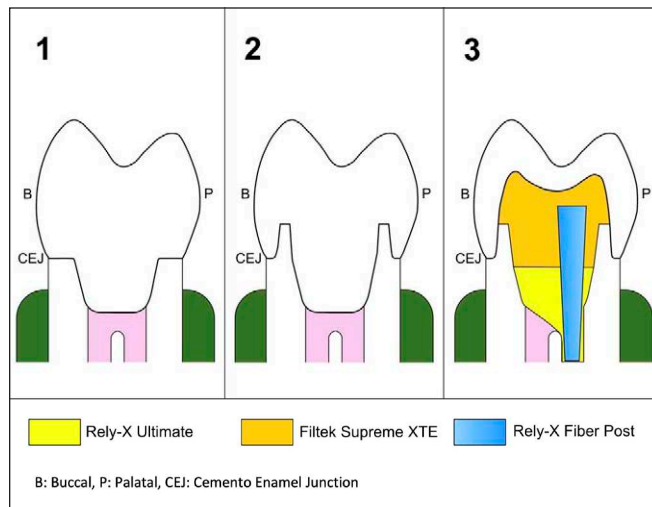


Figure 1. Experimental design of the study. Group 1 and 2 restorations were 5.5-mm thick from the mesio-distal sulcus to the cemento-enamel junction (CEJ) (without considering the 3-mm high endo-core for Groups 1 and 2). Group 3 crowns had a thickness of 2 mm from the mesio-distal sulcus to the resin core. The inner core of Group 3 specimens was 3.5-mm high from the CEJ including 2 mm of ferrule.

dimensions and with the same cusp occlusal angle, using one of the intact extracted maxillary first premolars as a master model in Cerec 4.0 software in Biogeneric Copy Design Mode.

Luting Procedures

Lined cavities of Groups 1 and 2, the core preparations of Group 3 and the intaglio surfaces of all the restorations were submitted to airborne-particle abrasion with 27 μ m aluminium oxide particles at about 0.2 MPa pressure for 5 seconds (Kavo EWL, Type 5423, Biberach, Germany), then rinsed and dried. The self-etch adhesive system (Scotchbond Universal Adhesive, 3M Oral Care) was applied on all prepared surfaces prior to cementation following the manufacturer's instructions. The bonding resin was left uncured, and the restoration was placed in a box, protected from light (Vivapad, Ivoclar Vivadent) until cementation. The tooth preparations were covered with a thin layer of dual-curing resin cement (RelyX Ultimate, 3M Oral Care) before seating the restoration. The restoration was placed first with manual pressure and then with the assistance of a specific ultrasonic device (Cementation Tip, EMS, Nyon, Switzerland). The plastic-coated ultrasonic tip was activated at the center of the restorative surface for 3 seconds to seat the workpiece into definitive position. After removal of excess cement, the restoration buccal, occlusal, and lingual surfaces were light cured for 90 seconds separately. Restorations were then immediately finished and polished, using

flame- and pear-shaped fine diamond burs (40 μ m, then 25 μ m grain size) (Intensiv Nos. 4205L, 4255, 5205L and 5255, Intensiv), and discs of decreasing grain size (Pop-On XT, 3M Oral Care) were used to polish their margins.

A summary of the products used in this study is listed in Table 1.

Thermo-mechanical Fatigue Loading

The stress test was carried out 24 hours after cementation in a chewing simulator (Chewing simulator CS-4, SD Mechatronik, Feldkirchen-Westerham, Germany) for TMCL. All specimens were subjected to 600,000 cycles with a 49 newton (N) eccentric occlusal loading force on the inner side of the buccal cusp. The force was applied with a metallic ball of 4 mm in diameter at 1.7 Hz frequency following a one-half sine wave curve. By having the specimen holder mounted on a hard rubber disc, a sliding movement of the tooth was produced between the first contact on an inclined plane of the buccal cusp and the central fossa (Figure 2). A total of 3000 thermocycles at 5°C to 50°C to 5°C were performed simultaneously.

Marginal Analysis

Before the fatigue test, as well as after completion of the loading phase, gold-sputtered epoxy resin replicas (Epofix, Struers, Rødovre, Denmark) were made from polyvinylsiloxane impressions (President light, Coltène, Altstätten, Switzerland). The dentin margins on the proximal side, below the CEJ, were observed. The tooth–cement interface (TC) was analyzed semiquantitatively by scanning electron microscopy (SEM) (Digital SEM XL20, Philips, Eindhoven, Netherlands), employing an established evaluation method. The margins were evaluated at a standard 200 $\times$ magnification; when required for assessment accuracy, higher magnifications up to 1000 $\times$ were used. The following evaluation criteria were considered: perfect adaptation (continuity), underfilling, overfilling, marginal opening, and tooth or restoration fracture. Results for marginal adaptation before and after the loading phase are expressed as percentages of “perfect adaptation” (defect-free). Percentages were calculated as the ratio between the cumulative distance of all segments showing the same morphological quality and the whole interface length.

Fracture Test

After fatigue, all groups were subjected to fracture load evaluation in a universal testing machine (Dyna-Mess, Stolberg, Germany) according to an established method. Each specimen was placed in

Table 1: Summary of Materials Used

Material	Brand Name	Chemical Composition	Elastic Modulus	Batch Number
Adhesive system	Scotchbond Universal Adhesive (3M-ESPE)	MDP, dimethacrylate, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators and silane		T02489
Resin cement	Rely-x Ultimate (3M-ESPE)	Base paste: methacrylate monomers, silanated fillers, initiators and stabilizers Catalyst paste: methacrylate monomers, fillers, pigments, initiators and stabilizers	7.7 GPa	T03823
Fiber glass post	Rely-x fiber post (3M-ESPE)	Glass fibers, composite resin	36 GPa	S14125
Composite resin	Filtek Supreme XTE Universal Restorative (3M-ESPE)	Methacrylates, fillers, initiators and stabilizers	10 GPa	S45474
RNC blocks	Lava Ultimate (3M-ESPE)	Highly cross-linked resin matrix reinforced by 80% by weight of silane treated nano zirconia-silica particles agglomerated to clusters (0.6-10 μm) and individual silane bonded nano silica or zirconia particles (< 20 nm)	15 GPa	S58320
Temporary resin	Fermit N (Ivoclar-Vivadent)	Methacrylates, dispersed silicon dioxide and copolymers, catalysts and stabilizers		S45172

Abbreviations: GPa, gigapascal; HEMA, 2-hydroxyethyl methacrylate; μm , micrometer; nm, nanometer.

a fixing device, and a controlled load was applied axially to the longitudinal axis of the root, using a stainless steel rod (diameter 5 mm). Pressure on the tip was applied in the middle of the occlusal surface at a crosshead speed of 1 mm/minute. Specimens were loaded until fracture and the maximum breaking loads were recorded in Newtons (N). The modes of fracture were analyzed by optical stereomicroscopy and classified as repairable restoration fracture, above the CEJ (type A), non-repairable/catastrophic vertical fracture, inside the restoration, below the CEJ (type B) and root fracture not involving the restoration (type C). Fractographic analysis by means of SEM was performed on selected specimens. To remove all the impurities, all fragments were cleaned in an ultrasonic 10% sodium hypochlorite bath for 3 minutes, rinsed with water, dried, and then fixed on the support for the microscope. The specimens were gold coated prior to the analysis with the SEM. Magnifications up to 2000 $\times$ were used to obtain higher definition of identified crack features in selected areas of interest. The overall direction of crack propagation

and failure origin(s) were systematically mapped for all specimens.

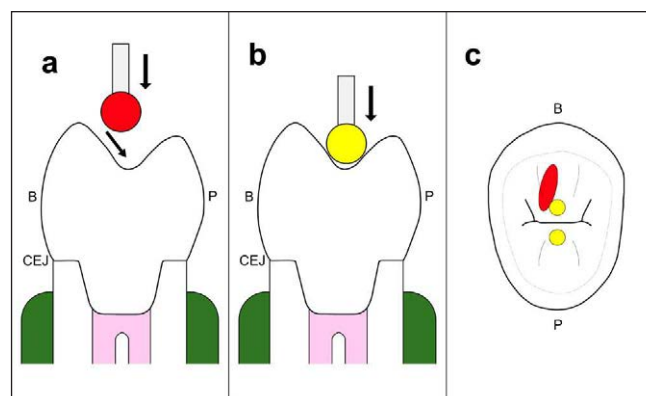


Figure 2. Schematic representation of (a) the sliding action of the ball indenter over the buccal cusp of the specimen during the Thermo-Mechanical Cycling Loading (TMCL) test and (b) the monocycle stress test. In the occlusal view (c), the contact of the sliding movement is in red and the contact points of the fracture strength test are in yellow.

Statistics

Marginal Adaptation

Data and statistical analyses were elaborated with R software, version 2.15.2 (R Foundation for Statistical Computing, Wien, Austria). The primary outcome was marginal adaptation, while the tested independent factors were the TMCL test (before and after fatigue) and the restoration technique (Groups 1 to 3). Because of the low specimen quantity in each group ($n=12$) and the uneven distribution of the primary outcome data, differences in marginal adaptation before and after stress were assessed by means of paired Wilcoxon tests. For the same reasons, differences in marginal adaptation between the techniques were estimated using Kruskal-Wallis tests. In the case of significant differences in marginal adaptation between the techniques, *post hoc* tests were used for pairwise multiple comparisons applying the Nemenyi test. To investigate the ferrule effect inside endocrowns, Group 1 data were compared to those of Group 2 (hypothesis a). For the comparison between endocrowns and crowns, the results of Groups 1 and 2 were paired and compared to those of Group 3 (hypothesis b). For all analysis, alpha level was set at 0.05.

Fracture Resistance

A Kruskal-Wallis test ($p>0.05$) was applied to assess the differences between the groups. Comparisons of the fracture load values of specimens were also achieved via Kaplan-Meier product limit estimation (OriginPro 8). Kaplan-Meier product limit estimation and fracture load values were established separately for the three groups. Plots of survival distribution were obtained for each group. The log rank method was used as test of the equivalence of survival times across groups. For the three groups, percentiles of the survival function (with lower and upper confidence limits) were calculated. The influence of the ferrule for endocrowns was assessed by comparing results of Group 1 and Group

2 (hypothesis a). The comparison between endocrowns and crowns (hypothesis b) was investigated by pairing results of Group 1 and Group 2 and comparing them to those of Group 3.

RESULTS

Marginal Adaptation

All the tested specimens survived the TMCL test. For each specimen, the percentage of perfect margin adaptation was calculated before and after the fatigue test. The results and statistical analysis are shown in Table 2 and Figure 3.

For all specimens, the results of perfect margin adaptation after stress were significantly lower than before loading. There was a significant difference between the experimental techniques in both before and after fatigue values. The highest median values were recorded for Groups 1 and 2, while Group 3 showed the lowest values. Groups 1 and 3 displayed the greatest difference of values for closed margins before and after the stress.

Integration of a ferrule (Group 2) into the classical endocrown design (Group 1) did not have any effect on marginal adaptation of the restorations.

Considering pairwise analysis of factors, endocrowns of Groups 1 and 2 performed better than crowns of Group 3 before and after stress even though degradation of the margins following the TMCL test was equivalent.

Fracture Resistance

The fracture load results are shown in Table 3. No significant differences were found between the groups with the Kruskal-Wallis test ($p>0.05$). Also, analysis of the Kaplan-Meier-plotted survival curves did not show any significant differences between the three groups (Figure 4). According to the Kruskal-Wallis test, the presence of a ferrule did not improve the load-bearing

Table 2: Results of Marginal Analysis Expressed as Median and Interquartile Percentage Ranges of Perfect Adaptation for the Three Groups ($n=12$), Before and After Loading

Group	Before ^a	After ^a	Diff (Af-Be)	p-value ^c
1	97 [94; 100] (3)	87 [79; 89] (3)	-12 [-16; -6]	<0.001
2	97 [94; 99] (3)	91 [85; 93] (3)	-5 [-8; -3]	<0.001
3	79 [73; 87] (1,2)	66 [60; 69] (1,2)	-13 [-25; -8]	<0.001
p-value	<0.001 ^b	<0.001 ^b	0.059 ^b	

^aData are medians [25th percentile; 75th percentile]. Numbers in parentheses represent the group(s) with which the concerned group is different according to the Nemenyi test.

^bKruskal-Wallis test.

^cWilcoxon paired test.

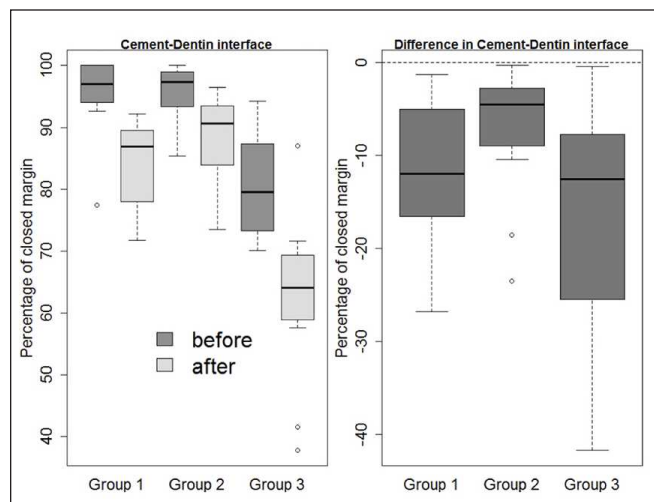


Figure 3. Box-and-whisker plots indicating percentages of "closed margins" for the three groups, before and after thermo-mechanical cycling loading (TMCL) fatigue loading.

capacity of the specimens of Group 2 compared to those of Group 1. Pairwise comparison of endocrowns and crowns did not show any difference in fracture resistance. The modes of failure are shown in Table 4. Most of the specimens experienced non-repairable fractures of type B, with a vertical fracture pattern which originated at the occlusal surface and progressed in an apical direction, splitting the restoration and the tooth mesio-distally (Fig. 5). As revealed by stereomicroscopic analysis, the fracture originated in all these cases under the loading area of the metallic indenter. However, some specimens of Group 3 (30%) fractured favourably, above the CEJ (type A fractures) (Figure 5). In all groups, a small percentage of restorations survived the test because of horizontal root fractures that left intact the tooth–restoration interface (type C fractures).

Table 3: Fracture Strength Test Results Expressed as Mean Fracture Loads and Standard Deviations, Median and Interquartile Values and Ranges

Fracture Load (N)				
Group	n	Mean $\pm$ SD	Median (IQ)	Range
1	12	894 $\pm$ 228	912 (699 - 1046)	444 to 1213
2	12	851 $\pm$ 145	845 (799 - 911)	568 to 1100
3	12	948 $\pm$ 318	1014 (782 - 1158)	386 to 1320
p-value		0.626 ^a	0.317 ^b	—

Abbreviations: IQ, interquartile; N, newton; SD, standard deviation.

^aLinear regression model

^bKruskal-Wallis test

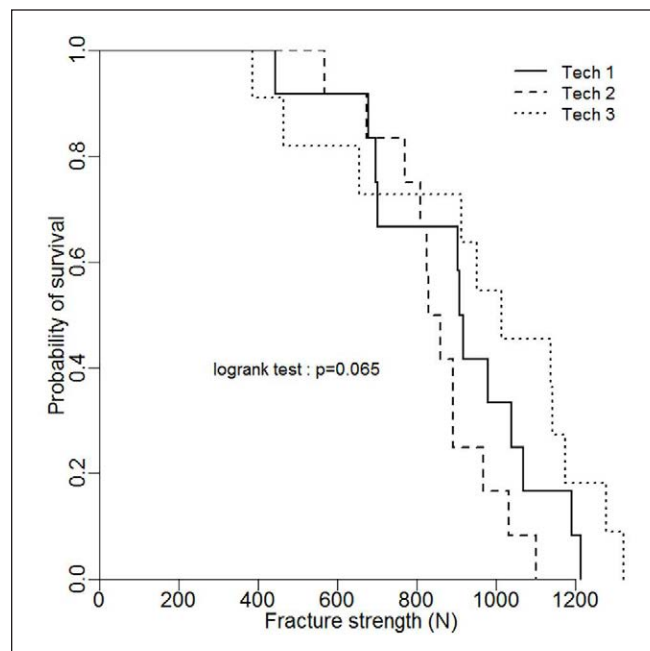


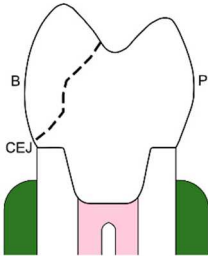
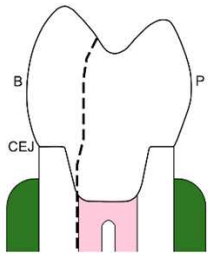
Figure 4. Kaplan-Meier plotted survival curves for the three groups.

DISCUSSION

In the present study, the investigators evaluated the impact of a ferrule on the marginal adaptation and fracture load of premolar endocrowns. To the best of the authors' knowledge, few studies exist on this topic. Results showed that the presence of a ferrule did not have any influence, either on marginal adaptation or on fracture resistance within the different endocrown restorations, meaning that hypothesis a was rejected.

Endocrowns performed better than crowns in terms of marginal integrity after cyclic stress, but their load-bearing resistance to the following monotonic loading was equivalent. Thus, hypothesis b was partially accepted. Severely destroyed first upper premolars were employed as specimens for this test because the efficacy of endocrowns to restore endodontically treated premolars still needs to be proved.²⁰ The worst clinical scenario was simulated by cutting the teeth at the CEJ and eliminating all of the anatomical crown. An endo-core length of 3 mm was chosen because it has been previously demonstrated that an extension of 3 mm inside the pulp chamber is sufficient to assure good marginal integrity and resistance to fatigue of premolar restorations.^{19,21}

An extracted upper premolar was used as the master model for crown shape and dimension of all milled restorations. An RNC CAD/CAM material (Lava Ultimate) was used for the restorations, because it is

Table 4: Types of Macroscopic Failure for the Three Groups			
Fracture Mode	Group 1 (#)	Group 2 (#)	Group 3 (#)
Type A 	0	0	4
Type B 	10	9	6

among the most commonly used materials in the chair-side fabrication of indirect restorations.

Specimens were fatigued by TMCL in a chewing simulator. A chewing force of 49 N was applied for 600,000 cycles at 1.7 Hz to reproduce *in vivo* masticatory conditions corresponding to approximately 2.5 years of clinical function in the premolar region.^{5,22,23} The sliding movement produced by the design of this test induced shearing stresses at the tooth-restoration interface, reproducing the effect of premolar guidance during the eccentric movements and exacerbating the testing conditions.²⁴ No artificial periodontium was employed around the roots, as materials used for this purpose tend to degrade and deform during the test and are therefore not standardizable. This could have negatively influenced the results of this test, as the periodontal ligament is a physiological shock absorber that plays a non-negligible role in *in vivo* conditions.^{19,25} Evaluation of the marginal adaptation of restorations was performed before and after the TMCL test. As expected, fatigue had a negative effect on marginal integrity for all specimens. In fact, it is well known

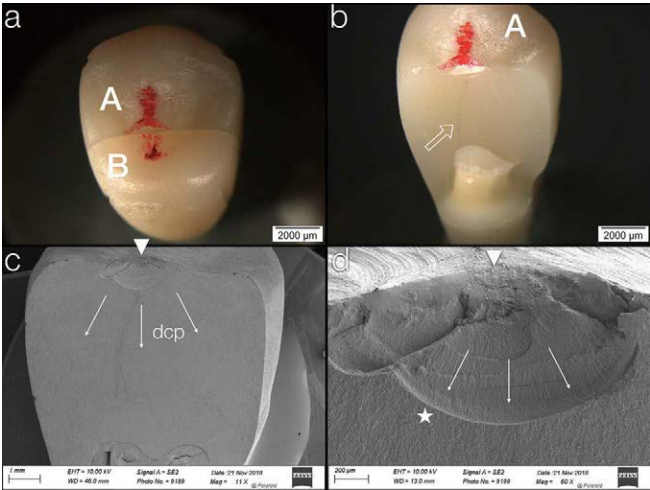


Figure 5. A typical type B catastrophic fracture. (a) Occlusal view from the stereomicroscope of the fractured specimen after repositioning of the two main halves. The red mark on the occlusal surface indicates the contact loading area of the monotonic strength test. (b) View of the fractured surface of fragment A. The crack started from the occlusal surface in a corono-apical direction and ended inside the root of the tooth with a smooth surface (fast fracture), causing the specimen to split. (c) Scanning electron micrograph (SEM) large view of the fractured surface. The white tip indicates the primary crack origin. Hackle lines on the surface indicate the direction of crack propagation (dcp, white arrows). (d) Details of the primary crack origin at higher magnification. Hackle lines are clearly visible.

that aqueous media, mechanical cyclic loading, and thermal stresses have a degrading effect on resin-bonded interfaces.²⁶⁻³⁰

The results of this study indicate that endocrowns of both Groups 1 and 2 performed better than crowns of Group 3 in terms of marginal integrity both before and after fatigue. This difference in outcomes even before the fatigue stress—just after polishing of the specimens’ margins—was not expected, as materials and luting conditions were the same for all specimens. The different geometry of the workpiece may have induced greater polymerization contraction stress at crown restoration margins compared to the endocrown interfaces, resulting in a lower quality of marginal adaptation. Further studies are necessary to confirm this hypothesis, as these results are in contrast with the trends observed in previous *in vitro* studies.^{19,31} In this study, degradation of margins (the difference between before- and after-fatigue values) was the same for all specimens, meaning that the technique used for restorations did not have an impact on the ageing of the adhesive interface.

All specimens survived the TMCL test. In the second part of the experiment, the specimens were stressed with a unidirectional monotonic load until fracture.

Results of Kruskal-Wallis tests and Kaplan-Meier survival curves displayed no significant differences for maximum static breaking loads among all three kinds of restored premolars. The similarity in the results within RNC endocrowns of Groups 1 and 2 (5.5 mm thick) and RNC crowns of Group 3 (2 mm thick) was expected despite the large discrepancy in the thickness of the restorations. In a previous study conducted by Chen and others,³² there was also no linear relation between fracture resistance and the thickness of RNC restorations. These results are also consistent with the outcomes of studies by Ramírez-Sebastià and others³³ and Rocca and others.⁵ Likewise, the fracture load test failed to provide evidence of any difference between endocrowns with and without a ferrule in the cavity preparation design. The importance of the thickness of the RNC endocrowns and the high loads needed to break them could explain the lack of effect of the two different preparations. It is reasonable to suppose that the similar mean load-bearing ability described for Groups 1 and 2 specimens reflects principally the final strength of the thick restoration more than the different cavity design. These results are not in agreement with previous *in vitro* studies in which ceramic molar endocrowns with ferrules outperformed those without them.^{4,18} Further *in vitro* studies are needed to clarify this issue.

In June 2015, 3M Oral Care removed the crown (and even endocrown) indication for its LAVA Ultimate CAD-CAM restorative product because of a higher-than-anticipated rate of debonding, while leaving the indication for inlays/onlays and veneers. In that notice, no details were included about the type of crowns involved (anterior vs posterior crowns, thickness of restoration), material of the core, or, above all, about the adhesive strategy involved in the debonded cases (type of cement, surfaces pre-treatment).⁵ In the present study, this product demonstrated good adhesive behavior in terms of marginal integrity after fatigue of both LAVA crowns and endocrowns, without any final debonding. Magne and others³⁴ had previously investigated fatigue behavior after accelerated fatigue testing (stepwise loading) of molar crowns (2.5 mm and 1.5 mm thick) and endocrowns (3.5 mm thick) fabricated with the same RNC material and bonded to dentin and composite cores. No ferrule was included in the tooth cavity design. All restorations survived the normal ranges of posterior masticatory forces without any debonding. Later, the same group obtained similar good results in terms of adhesive and mechanical properties after fatigue for RNC molar crowns bonded to dentin, and their performances were equivalent to lithium-disilicate reinforced ceramic counterparts.^{35,36} Several other *in vitro* studies showed excellent

mechanical properties of RNC crowns and endocrowns bonded to resin dentin analogues.^{5,37,38} To the best of the authors' knowledge, no clinical trials exist at present that evaluate LAVA Ultimate crowns bonded to natural tissues or conventional composite resin cores. A recent randomized clinical trial on posterior implants restored with zirconia abutments and RNC crowns encountered a high rate (80%) of premature bonding failure, discouraging the use of this material for crowns bonded to rigid zirconia abutments.³⁹

With regard to the mode of fracture, almost all specimens fractured in a catastrophic way (type B fracture) because of vertical restoration-root fractures (Figure 6). In these cases, stereomicroscopic analysis of the fractured specimens revealed that the origin of the primary crack was always placed under the main occlusal contact area of the loading ball indenter. The crack front propagates in a vertical corono-apical direction without obstacles and creating smooth fractured surfaces. Nonetheless, some Group 3 crowns showed more favorable fracture patterns. Fractographic analysis showed that in these cases the crack was deviated during its run at the adhesive interface between the crown and the core (Figure 5).

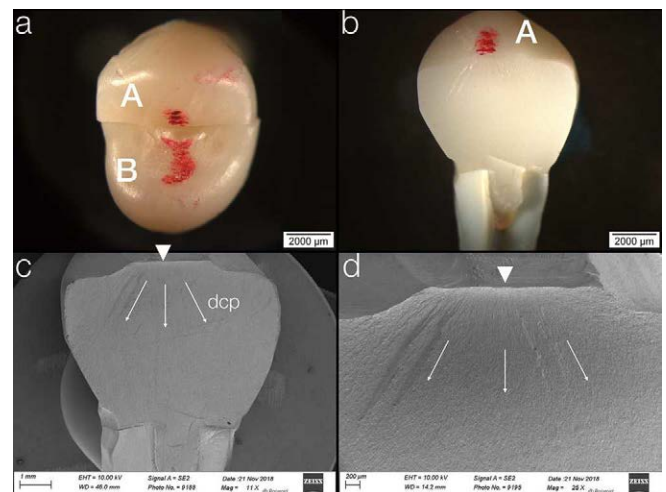


Figure 6. An example of type A fracture of a crown. (a) Occlusal view from the stereomicroscope of the fractured specimen after repositioning of the two main halves. The red mark on the occlusal surface indicates the contact loading area of the fracture strength test. (b) Fracture started from the occlusal surface and had a corono-apical direction. The empty arrow indicates the trail of the metallic ball after the crack occurred. A deviation of the crack happened at the adhesive interface between the crown and the core. The fracture broke a portion of the resin core and then passed through the interface between the crown and the tissues of the core. These fractures were classified as repairable. (c) SEM large view of the fractured surface. The big white tip indicates the primary crack origin. White arrows indicate the direction of crack propagation, (dcp, hackle lines). (d) Details of the primary crack origin at higher magnification. The white star indicates an arrest line.

CONCLUSIONS

Under the conditions of this *in vitro* study, RNC endocrowns that included a ferrule in the geometry of the cavity did not show better marginal adaptation after fatigue or improved fracture resistance after monotonic loading than endocrowns without a ferrule. Almost all specimens fractured in a catastrophic way, under the CEJ. Further *in vitro* studies and clinical trials are needed to confirm these results.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Commission cantonale d'éthique de la recherche (CCER) in Geneva Switzerland.

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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Effects of Microabrasion Prior to In-office Bleaching on Hydrogen Peroxide Permeability, Color Change, and Enamel Morphology

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Clinical significance

A waiting time of 7 days after performing microabrasion (MA) before performing in-office (IO) bleaching decreases the potential for hydrogen peroxide to reach the pulp chamber.

SUMMARY

Purpose: This study evaluated hydrogen peroxide (HP) diffusion within the pulp chamber, as well as color change and the surface morphology of teeth subjected to various microabrasion (MA) protocols associated or not with

in-office (IO) bleaching. Methods: Forty sound premolars were randomly divided into the following four groups (n=10): no treatment (NC); IO bleaching only; IO immediately after MA (IMA), and IO seven days after MA (7MA). After treatments, the HP concentration ($\mu\text{g/mL}$) within the pulp chamber was determined using ultraviolet-visible (UV-Vis) spectrophotometry. The color change (ΔE^*) was evaluated using the digital spectrophotometer before and 1 week after bleaching. The surface morphology was evaluated by scanning electron microscope (SEM). Data from each test were submitted to one-way ANOVA and Tukey tests ($\alpha=0.05$). **Results:** All experimental groups exhibited higher HP concentrations compared to the NC group ($p<0.00001$). However, higher amounts of HP were observed for the IMA group compared to the IO and 7MA groups ($p<0.00001$). No significant difference in color

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change was observed among the groups ($p<0.001$). Pronounced grooves in enamel were found in the IMA and 7MA groups. However, enamel erosion areas were observed only in the 7MA group. **Conclusions:** The association between MA and IO bleaching could significantly affect the amount of HP inside the pulp chamber. Therefore, it is highly recommended to wait for 1 week after MA procedures before performing IO bleaching.

INTRODUCTION

Dental fluorosis is an enamel defect that occurs due to excessive chronic fluoride intake during the tooth formation process.^{1,2} The structural changes caused by this anomaly generate porosity and reduced mineral content, causing visual effects on the enamel.^{3,4} This effect can result in esthetic compromises ranging from thin white lines to extensive opaque areas. Indeed, depending on the severity of the porosity, these spots can acquire a yellowish or brownish appearance.⁵⁻⁸ The negative esthetic effects of fluorosis can also impact patient quality of life.^{9,10} Therefore, in the last decade, demand by patients seeking treatment to improve their smiles' esthetics has increased.

Many techniques can improve the esthetic appearance of tooth stains caused by fluorosis. A recent systematic review¹¹ examined the aspects of minimal intervention and established that the most effective current treatment options for dental fluorosis include tooth bleaching, microabrasion (MA), or a combination of both treatments.

MA is a conservative, simple treatment aimed at removing stains or defects restricted to the enamel through chemical and abrasive action.^{12,13} The microabrasive agent facilitates the controlled removal of thin enamel layers (varying from 20 to 200 μm). In this sense, the amount of removal depends on the acid concentration, the abrasiveness of the particles, the application time, and the pressure exerted on the enamel during the procedure.¹³⁻¹⁵ After enamel MA, teeth may develop a yellowish appearance due to the reduction of the enamel's thickness, which makes the underlying dentin more evident. Therefore, bleaching has become an option for improving the esthetic appearance of teeth subjected to MA. This is one of the main reasons several authors indicate a combined treatment (MA and bleaching).^{9,16-18}

Unfortunately, when this combined treatment is required, tooth sensitivity is the most common adverse effect patients report.^{16,17} Given that tooth

Table 1: Description of the Materials (Manufacturers) and Composition of the Products Used in This Study

Material (Manufacturers)	Composition
Opalustre [Ultradent, South Jordan, UT, USA]	6.6% Hydrochloric acid and silicon carbide microparticles with particle size from 20 to 160 μm
Opalescence Boost 40% [Ultradent, South Jordan, UT, USA]	40% hydrogen peroxide (HP), 0.11% Fluor ions (1000 ppm), and 3% potassium nitrate

sensitivity is related to the amount of hydrogen peroxide (HP) that reaches the pulp chamber,¹⁹ several authors have hypothesized that the enamel thickness reduction after MA could be partly responsible for increased tooth sensitivity in patients submitted to bleaching procedures.^{9,16,17} Hence, once the amount of mineralized structure and thickness is reduced,¹³⁻¹⁵ it may be important to remineralize the enamel surface before applying an in-office (IO) bleaching gel to avoid an increased HP diffusion into the pulp chamber.

Therefore, this *in vitro* study was aimed at quantifying the permeability of HP within the pulp chamber and evaluating color change and surface enamel morphology in human teeth submitted to various protocols that combine MA and IO bleaching. The null hypotheses tested were that MA performed prior to IO bleaching (1) will not increase concentration of HP inside the pulp chamber, (2) will not change the surface morphology, and (3) will not interfere in the bleaching efficacy of an IO bleaching product.

METHODS AND MATERIALS

Forty sound upper premolars were donated by patients who underwent tooth extraction at the local university for orthodontic reasons. Specimen enamel analysis was performed using a stereomicroscope (Lambda LEB-3, ATTO instruments, Hong Kong, China) at 10× magnification. Specimens with morphological alterations or the presence of enamel cracks were excluded. The products used are described in Table 1.

Sample size calculation

The study's primary outcome was the amount of HP that reached the pulp chamber. According to a previous study,²⁰ the amount of HP inside the pulp chamber in the groups submitted to IO bleaching was 0.28, whereas it was 0.004 μg/mL for the control group (without IO bleaching). To achieve a power of 80% and detect significant differences at the 5%

Table 2: Data Points for the Calibration Curve

H ₂ O ₂ Data For Each Point		Solutions Required to Obtain 3000 µL for Each Point for the Calibration Line				
H ₂ O ₂ Weight (µg)	H ₂ O ₂ Concentration (µg/mL)	Acetate Buffer Solution (µL)	46.15 µg/mL H ₂ O ₂ Solution (µL)	Peroxidase (µL)	Leuco Crystal violet (µL)	Deionized Water (µL)
1.0704	0.356	75	25	50	100	2750
0.8563	0.285	80	20	50	100	2750
0.6422	0.214	85	15	50	100	2750
0.4281	0.143	90	10	50	100	2750
0.3425	0.114	92	8	50	100	2750
0.1710	0.057	96	4	50	100	2750
0.0855	0.029	98	2	50	100	2750
0.0000	0.000	100	0	50	100	2750

level, the minimal sample size was nine teeth in each group to detect differences of 0.2 µg/mL among the tested groups. However, considering the possible loss of samples, 10 teeth were used for each group.

Specimen preparation

The roots of the teeth were removed approximately 3 mm apical to the cemento-enamel junction using a low-speed diamond disk (Isomet 1000, Buehler, Lake Bluff, USA). The pulp tissue was removed and rinsed with ultrapurified water. Access to the pulp chamber was expanded carefully with a round bur (#1014, KG Sorensen, São Paulo, SP, Brazil) to allow 25 µL of solution to be introduced into the pulp chamber using a micropipette (LabMate Soft, HTL Lab Solutions, Warsaw, Poland).

Obtaining the study calibration curve

The study used analytical products without prior purification, and all solutions were prepared using ultrapurified water obtained from a Millipore Milli-Q system (MS2000, Gehaka, SP, Brazil). First, a standard reference line was plotted using a 5.000 µg/mL stock solution prepared from a concentrated solution (34%-36% HP, LABSYNTH, Diadema, SP, Brazil); this solution was diluted in an acetate buffer solution (pH=4.5) and calibrated using traditional methods. The solution was titrated with a potassium permanganate solution to determine its analytical grade and actual solution concentration.²¹ Based on this verified initial concentration, serial volumetric dilutions of 0.000-0.356 µg/mL were performed to plot the line of work (Table 2).²² The known HP concentrations were added to the glass tubes and placed in a Varian Cary 50 UV-Vis (ultraviolet-visible) spectrophotometer (Varian, Palo Alto, CA, USA). This procedure yielded a standard reference

line for the extrapolation of the study samples' results ($R=0.998$).

Experimental groups and treatment protocols

The forty sound premolars selected previously were allocated randomly into four groups (n=10) according to the treatments. In the group that received no treatment (NC), no bleaching or MA procedures were performed. In the IO bleaching group (IO), only an IO procedure (Opalescence Boost 40%; Ultradent Products, South Jordan, UT, USA) was applied. In the third and fourth groups, MA (Opalustre, Ultradent) was performed prior to IO bleaching (Opalescence Boost 40%; Ultradent). However, in the third group, IO bleaching was applied immediately after MA (IMA), whereas in the fourth group, IO bleaching was applied seven days after MA (7MA). In this latter group, specimens were stored in artificial saliva during this period.

For the third and fourth groups, the MA treatment was performed using Opalustre in accordance with the manufacturer's recommended protocol. The product was applied to the enamel surface for 60 seconds by active application with a rubber cup (OpalCups, Ultradent). The OpalCups were used in a low-speed handpiece with circular movements at 500 rpm and intermittently applied low pressure similar to a prophylaxis procedure. The procedure was performed twice per each tooth. Finally, it was washed and air dried. For all treatment protocols, a single calibrated and experienced operator, blinded to the assigned groups, was responsible for the material application.

Hydrogen peroxide quantification inside the pulp chamber

For all groups, the specimens were vertically fixed to a wax plate with the occlusal surface toward the

plate. Before the bleaching agent was applied, the buccal surface of each specimen was isolated by applying a light-cured resin barrier enclosing an area of 8 mm² (OpalDam, Ultradent). A 25-μL aliquot of acetate buffer (pH = 4.5) was inserted into the pulp chamber of each tooth to absorb and stabilize any HP that might penetrate the pulp chamber.

In the experimental groups, the bleaching treatment was performed using Opalescence Boost 40% (Ultradent). The gel was applied to the enamel in a single 20-minute session. Following the bleaching procedure (which was not performed in the NC group), the acetate buffer solution inside each specimen's pulp chamber was removed using a mechanical micropipette and transferred to a glass tube. This procedure was performed by rinsing each specimen's pulp chamber four times with 25 μL of acetate buffer and transferring this solution to the same glass tube. Thereafter, more ultrapurified water (2.725 μL) was added to the glass tube along with 100 μL of 0.5 mg/mL (Leuco Crystal Violet, Sigma Chemical Co, St Louis, MO, USA) and 50 μL of 1 mg/mL horseradish peroxidase enzyme (Peroxidase Type VI-A, Sigma Chemical). This procedure was repeated separately for each specimen. The resulting solution had a violet color with a maximum absorbance peak at 596 nm, which was measured using a Varian Cary 50 UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA). According to Beer's Law, absorbance corresponds directly to concentration. Therefore, HP concentration (μg/mL) was determined by comparing it with the calibration curve already obtained.

Color change evaluation

Color change was measured before and 1 week after bleaching treatments using a digital spectrophotometer (VITA Easyshade Advance 4.0, Vita Zahnfabrik, Bad Säckingen, Germany). During this period, specimens were immersed in artificial saliva, with daily changes at a controlled temperature of 37°C.

To standardize the position of the spectrophotometer, impressions were taken using a condensation silicone paste (Coltoflax and Perfil Cub Kit, Vigodent, Rio de Janeiro, RJ, Brazil). A 6-mm diameter window referring to the tip of the spectrophotometer was created in the middle-third of each specimen's buccal surface using a metal device. The color parameters (L^* , a^* , and b^*) were recorded using the tip of the device, which was inserted into the impression guide. The L^* value represented the lightness, values for which varied from 0 (black) to

100 (white). The a^* value represented the color along the red–green axis, and the b^* value represented the color along the yellow–blue axis. Color change (ΔE^*) was calculated based on the difference between the specimen before (baseline) and 1 week after bleaching for each group, using the following formula from CIELab 1976: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Enamel surface morphology evaluation

After the previous test, three fragments were obtained from each group to evaluate enamel surface morphology. After being cut, each sample was cleaned in an ultrasonic bath with a frequency of 42 kHz (Cristófoli Ultrasonic Washer, Cristófoli, Brazil) and dehydrated in a desiccator containing colloidal silica for 24 hours at 37°C and sputter-coated with 10 nm of gold before being taken to a low-vacuum scanning electron microscope (SEM). To standardize image acquisition, three pictures of each specimen were acquired at a magnification of 10,000× in the secondary electron mode at 15 kV (MIRA3, TESCAN ORSAY HOLDING, Warrendale, PA, USA). The first image was taken in the center, and the other two images were taken 0.3 mm to the left and right of the first image. A technician who was blinded to the experimental conditions evaluated all images and selected the most representative one.

Statistical analysis

Before submitting the data for analysis using the appropriate statistical test, the Shapiro–Wilk test was performed to assess whether the data followed a normal distribution. Bartlett test for equality of variances was performed to determine whether the assumption of equal variances was valid. After evaluation of the normal distribution, the data obtained regarding HP concentration (μg/mL) and color change (ΔE^* measurements) were statistically analyzed using one-way ANOVA and Tukey tests ($\alpha=0.05$). The enamel surface morphology was only evaluated qualitatively.

RESULTS

Hydrogen peroxide quantification inside the pulp chamber

One-way ANOVA revealed statistically significant differences among groups for HP concentration ($p=0.0001$; Table 3). A lower amount of HP was detected inside the pulp chamber of the NC group when compared to the bleaching groups ($p<0.00001$; Table 3). However, the highest and only significant

Table 3: Means and Standard Deviations of HP Concentration ($\mu\text{g/mL}$) Detected inside the Pulp Chamber and ΔE^* Measurements after Treatment

Experimental Groups	Hydrogen Peroxide (HP) Concentration ($\mu\text{g/mL}$)	ΔE^* Measurements
No treatment (NC)	0.0001 ± 0.0001 A	2.5 ± 0.7 b
Only in-office (IO)	0.32 ± 0.04 B	7.0 ± 2.7 a
IO immediately after microabrasion (IMA)	1.05 ± 0.43 C	7.6 ± 3.1 a
IO 7 days after microabrasion (7MA)	0.41 ± 0.15 B	5.8 ± 2.1 a

**Identical uppercase, lowercase, or superscript letters in each column indicate statistically similar means (One-way ANOVA and Tukey test, $\alpha=0.05$).*

amount of HP was observed for the IMA group compared to IO and 7MA groups ($p < 0.00001$; Table 3).

Color change evaluation

One-way ANOVA revealed statistically significant differences among groups for color change ($p = 0.001$; Table 3). A lower, imperceptible color change was observed in the NC group when compared to the other groups ($p = 0.001$; Table 3). All bleaching groups showed similar ΔE values ($p > 0.05$; Table 3).

Enamel surface morphology evaluation

Differences in surface morphology were observed between the NC group and the bleaching groups (Figure 1). Mild superficial alterations, irregularities, and slight demineralization was observed in enamel in the IO group. Pronounced grooves were observed on the enamel surfaces in the groups that underwent MA. However, areas of erosion on the enamel surface were observed only in the IMA group (Figure 1).

DISCUSSION

The presence of HP inside the pulp chamber of the groups submitted to bleaching was expected, because the active HP molecules used in the bleaching agents have a low molecular weight. HP penetrates and interacts with organic components present in the dental structure, thus promoting a color change.¹⁹ This is why HP has the capacity to diffuse inside the tooth, reaching the pulp tissue within 15 minutes after application.²³⁻²⁵

However, the IMA group showed the highest HP values inside the pulp chamber when compared to other IO bleaching groups. This led us to partially reject the first null hypothesis. The MA product used on the enamel surface contains hydrochloric acid and abrasive particles. The former is responsible for causing significant erosion in the enamel surface, which is followed by wear due to the rubbing application of abrasive particles leading to a removal of a thin layer of enamel (20-200 μm).¹³⁻¹⁵ Previous studies showed that the thickness of the enamel played an important role in the passage of HP into the pulp chamber.²⁶⁻²⁸ Thus, at first glance, a reduction in enamel thickness where the MA procedure was applied might be responsible for the higher amount of HP inside the pulp chamber. However, similar enamel reduction should have been expected when the same MA procedure was applied, as for IMA and 7MA groups, and only the former showed higher HP values inside the pulp chamber.

As is known, the MA procedure promotes significant changes in the proportion of organic and inorganic components, mainly by modifying the interprismatic spaces at the enamel surface,^{29,30} which may potentially alter the permeability of the enamel surface of dental tissues,²⁶ and, consequently

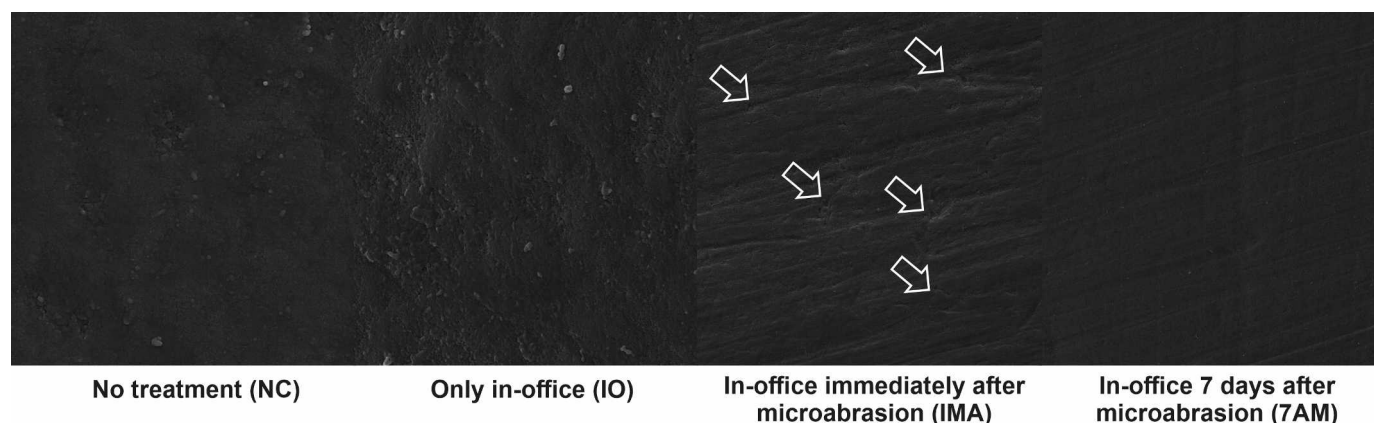


Figure 1. Morphology of the enamel surface obtained via scanning electron microscopy at 10,000 $\times$ magnification.

may facilitate HP diffusion along the tooth structure. For both groups in which MA procedures were applied (IMA and 7MA), it was possible to see pronounced grooves on the enamel surface when compared to the control, and only IO groups led us to reject the second null hypothesis.

There were substantial differences observed in the SEM images for both MA groups. The presence of more evident erosive zones in the enamel surface was observed for the IMA group. This indicated an increasing susceptibility of “freshly” microabraded enamel to greater surface demineralization, which produced eroded areas³¹⁻³³ primarily when IO bleaching was applied immediately after the MA procedure. Therefore, the more pronounced alterations in the enamel surface, combined with enamel permeability, could have made the enamel more susceptible to bleaching agent penetration in the IMA group.

On the other side, a considerable reduction in the amount of HP inside the pulp chamber in the 7MA group should be attributed to the remineralizing effect due to the specimen storage in artificial saliva at a controlled temperature for a week before performing IO bleaching. Saliva and its components might play an important role in maintaining the integrity of dental tissues when they are prone to mineral loss.³⁴ Some studies have speculated that the interaction of saliva with microabrasive components can be significant and might be related to the compression of silica particles in enamel when performing the MA. This results in tricalcium silicate (Ca_3SiO_5), which indicates the formation of a new layer of apatite crystals in the microabraded enamel.^{35,36}

Therefore, based on the results of the present study, the increased amount of HP inside the pulp chamber observed in the IMA group should be more related to increased permeability of the enamel outer layer after the MA than due to enamel thickness reduction observed after the MA procedure.

Although several changes occurred in the enamel surface, mainly when MA procedures were performed, no detrimental effects were observed in terms of bleaching effectiveness. All groups exposed to IO bleaching produced a color change of 5.8-7.0 ΔE^* , regardless of the surface treatment performed. Therefore, the third null hypothesis that the use of MA might affect color changes was rejected.

One of the current study's limitations was the use of only one IO bleaching and one microabrasive product. Several characteristics could influence the

final results of IO bleaching, including pH, application technique, viscosity, and chemical stability,^{22,25,26} as well as the microabrasive system, the effects of which rely on the concentration and pH of the acid used, the type of abrasive included in its composition, instrumentation time, applied force, application mode, and revolutions per minute. All these situations are related to the changes observed in the microabraded enamel.

However, the decision to evaluate the IO bleaching technique was based on thinking in a scenario where a single appointment of MA followed by IO bleaching could be carried out.

Another limitation of the present study was the bleaching technique applied. Taking in consideration that at-home bleaching is a popular and safe technique, mainly because low concentration HP is used, at-home bleaching could be considered the more desirable when the MA procedure is performed. In the other side, the decision to evaluate IO bleaching, instead of at-home bleaching, was based on a single appointment where MA followed by IO bleaching could be carried out. Therefore, future studies using different MA products and techniques, as well as combined with at-home bleaching, need to be done to confirm the hypothesis.

CONCLUSIONS

Based on the results obtained in this study, the three null hypotheses tested were rejected; using an MA product immediately before IO bleaching increased HP diffusion into the pulp chamber and produced significant alterations in the enamel surface. However, these effects did not interfere with the efficacy of the bleaching agent in terms of color changes. Clinicians should avoid the MA procedure immediately prior the use of an IO bleaching product.

Avoiding a combination of MA and IO bleaching in a single session is recommended due to increased HP levels inside the pulp chamber and a greater erosive potential on the enamel surface. Changes in the tooth enamel do not interfere with the color changes exhibited by teeth submitted to IO bleaching.

Conflicts of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this manuscript.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guide-

lines and policies of Institutional review board of National University of Colombia. The approval code issued for this study is HERMES 45686.

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Effect of Cervical Lesions on Fracture Resistance and Failure Mode of Maxillary Central Incisors Restored with Fiber Posts and Complete Crowns

D Santos Pantaleón • FM Valenzuela • BR Morrow • CH Pameijer • F García-Godoy

Clinical Relevance

In the presence of a cervical cavity, placement of a fiber post is recommended to strengthen a maxillary central incisor. This increases the fracture resistance of the tooth, leading to a more favorable prognosis of the completed restoration.

SUMMARY

Purpose: To investigate the effect of a cervical cavity extending 1 mm apical to the cemento-enamel junction (CEJ) on fracture resistance

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and failure mode of maxillary central incisors that have been treated endodontically, present with complete and incomplete ferrules, and are restored with and without a fiber post.

Methods and Materials: 50 intact human maxillary central incisors were divided into five groups (n=10): CG (control group) 6-mm ferrule height, no cervical cavity, and without post; (CO) 6-mm ferrule height without post, with a cervical cavity (access to root canal and cervical cavity restored with composite resin), cervical cavity; and post with ferrule heights of 1 mm (CP1), 2 mm (CP2), and 6 mm (CP6) restored with fiberglass post and composite resin core. After complete metal crowns were cemented on all specimens, they were subjected to thermal cycling (6000 cycles, 5°C/55°C),

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followed by immediate testing of fracture resistance. After failure, the specimens were sectioned buccolingually to evaluate and identify the mode of failure. The data were analyzed with an analysis of variance (ANOVA) and the Student–Newman–Keuls multiple comparison tests ($\alpha=0.05$).

Results: A 1-mm ferrule height (CP1) fracture resistance was significantly lower (531 ± 125 N) compared to the 6-mm ferrule height (CP6) (769 ± 175 N) ($p < 0.05$). With respect to the groups with similar residual dentin, with and without a cervical cavity, CG (667 ± 119 N) and CO (668 ± 119 N), the presence of a post (CP6) increased the resistance to fracture, although no statistically significant difference was demonstrated. Partial decementation was observed in all specimens of CG and CP6, in nine of CP1 and CP2, and in three in CO. Root fractures occurred in 23 specimens. The root surface was exposed 2 mm below the CEJ to simulate bone level. Propagation of subosseous cracks occurred in four specimens in CG and CP2, in seven specimens in CP6, in two specimens in CP1, and in six specimens in CO. All were considered catastrophic failures.

Conclusions: Within the limitations of this study it is suggested that, when restoring an endodontically treated maxillary central incisor that has a cervical lesion and needs to be restored with a complete crown, a fiber post is cemented to improve fracture resistance.

INTRODUCTION

Complete crown restoration of endodontically treated maxillary central incisors is challenging due to extensive loss of tooth structure, which may have been caused by caries, fracture, endodontic access, and instrumentation and preparation for post and crown.¹⁻³ The greater the loss the more it negatively affects the resistance to fracture.⁴⁻⁸ Furthermore, anterior teeth are stressed by horizontal forces, exposing them to a higher risk of biomechanical failures.^{9,10} Quantity and location of the remaining dentin appears to be the most important factor in predicting the prognosis of a tooth.^{11,12} If there is enough remaining dentin for the preparation of a complete ferrule, the outcome will be more favorable.¹³ By definition, a circumferential ferrule is formed by four axial dentin walls that are 2-mm high from the cervical finish line to the coronal junction.¹⁴⁻¹⁹ Several publications reported on the protective effect this offers on the final restoration as it

reduces the concentration of stresses, thus decreasing clinical failures such as root fracture and/or decementation of the post.²⁰⁻²²

However, for various reasons, such as caries, erosion, abrasion or fracture, a circumferential ferrule is not always possible.²³ This affects the prognosis of a restoration.²⁴ Furthermore, the superiority of one partial ferrule over another depends on the height, number, and location of the remaining walls.²⁵⁻³⁰ Because the ferrule effect is not entirely understood, it has been suggested that more studies are necessary.³¹ In particular, maxillary incisors with cervical lesions and endodontic treatment has received little attention.³² It is this type of defect that affects the thickness of the facial wall, which compromises the integrity of the ferrule.³² When a cervical lesion involves the root, it is commonly associated with gingival recession.³³ As a result, the preparation of the cervical shoulder margin has to include the lesion on the root surface, which has less dentin thickness due to the conicity of the root.^{14,34}

The thickness of the dentin wall plays an important role in the function of the ferrule, which most studies have not considered.³² It has been advised to keep enough facial dentin in maxillary anterior teeth to counteract horizontally directed forces, as at least a 1-mm thick dentin ferrule may resist functional loads and prevent fracture.³⁵ However, there is insufficient research information available that supports the effectiveness of this ferrule. In reviewing the literature, the work of Tjan and Whang³⁵ can be considered a pioneering study with reference to the thickness of the facial wall of remaining dentin in maxillary central incisors at the level of the cemento–enamel junction (CEJ). The thickness was 1, 2, and 3 mm in combination with cast posts, without ferrule effect (no cemented crown). The authors compared fracture resistance under horizontal force, showing that although there were no statistically significant differences, the 1-mm thick facial dentin wall was more prone to fracture than to decementation. It should be noted that since the samples had no ferrules, and no complete crowns were cemented, the results should be interpreted with caution. Joseph and Ramachandran³⁶ demonstrated that more than 1-mm thickness is required in the facial dentin wall to prevent root fracture. Furthermore, a 2-mm high dentin ferrule improved fracture resistance. Recently, Fontana and others³⁷ suggested that when a ferrule thickness is 1 mm, it is more appropriate to use a fiber post. Naumann and others³⁸ observed that in excessively widened

teeth it is not possible to establish a ferrule, because the preparation will cause even more loss of tooth structure, resulting in a very thin wall or no wall at all.

The authors found no studies in the literature regarding the effect of cervical lesions on fracture resistance in the maxillary endodontically treated central incisor restored with a complete crown, except for a single study using the direct resin composite technique with or without a fiber post.³⁹ The purpose of this study was to evaluate the effect of the cervical cavity extending 1-mm apical to the CEJ, with variations in height of the remaining coronal structure on fracture resistance and the mode of failure of endodontically treated maxillary central incisors and restored with a fiber post. The null hypothesis was that the placement of a fiber post in maxillary crowned central incisors and in the presence of a restored facial cervical lesion will not affect the fracture resistance.

METHODS AND MATERIALS

Specimens and Preparation of Root Canal

Fifty intact human maxillary central incisor teeth recently extracted for periodontal reasons were stored in a 0.9% physiologic saline solution (Laboratorios ALFA, Santo Domingo, Dominican Republic) with 0.1% Chloramine T (Sigma Aldrich, St. Louis, MO, USA) at room temperature. The teeth were cleaned to remove soft tissue and debris and were examined under 3.5× magnification (Double lens head-wearing, Gerleek, YTOM, China) for defects. X-rays were taken to verify the absence of fractures, internal root resorption, and obstructions or endodontic filling material. The teeth were stored at room temperature and were used within 3 months post-extraction.

The root lengths (13.8 ± 0.9 mm) and the buccolingual diameters (6.6 ± 0.4 mm) at the CEJ were measured with an electronic caliper (Mitutoyo Absolute 573-751, America Corp, Aurora, III, USA). The teeth were divided into five experimental groups of 10 teeth each ($n=10$). The power test on the data was "Power of performed test with $\alpha=0.050$: 0.998." Therefore, the sample size was sufficient. The nature of variability existing in all tissue is such that data must vary, and standard deviations will be relatively large.

The preparation of the endodontic access cavity was made with a round bur (No. 6801.31.014, Brasseler, Savannah, GA, USA), outlining a triangular cavity. All teeth received conventional end-

odontic treatment using manual instrumentation with files up to #40 (Dentsply Sirona, York, PA, USA), irrigation with 3% sodium hypochlorite (ChloridCid; Ultradent Products Inc., South Jordan, UT, USA), lateral condensation with gutta-percha (Dentsply Sirona), and use of a sealing cement (AH 26, Dentsply Sirona).

Preparation and Ferrule

In all teeth, except the control group (CG), the cervical cavity was prepared as follows. A round diamond bur (no. 6801.31.014, Brasseler) was used to prepare the cavity characterized by a gingival wall parallel to the CEJ and extending 1 mm apical to it, mesial and distal walls parallel to the long axis of the tooth, and an incisal wall following the contour of the incisal edge.⁴⁰ Subsequently, with a cylindrical carbide bur (SSW no. FG-558, SSW, New Jersey, NY, USA), the dimensions of the cavity were defined: height = 3 mm, width = 3 mm, and depth = 2 mm. Crown preparation was performed with a super coarse round-end taper FG diamond bur (no. 5856.31.018 Brasseler). Preparations were done under copious water cooling. The reduction of the crown was performed according to the height of the pre-established remaining walls in the experimental groups, following the contour of the CEJ as a reference.⁴¹ The location of the facial cervical finish line of the preparation followed the contour of the cervical cavity and was located 1 mm apical to the CEJ, while it was located at the level of the CEJ in the remainder of the tooth. The finish line served as a reference for the linear measurement of the remaining coronal heights in each specimen. While the CG had no cervical cavity, the location of the finish line was similar to the other groups. Thus, the following five experimental groups were established (Fig. 1): CG (6-mm ferrule height, no cervical cavity, and no post), CO (6-mm ferrule height, with a cervical cavity), CP1 (1-mm ferrule height, cervical cavity, and post), CP2 (2-mm ferrule height, cervical cavity, and post), and CP6 (6-mm ferrule height, cervical cavity, and post). Table 1 presents the materials, composition, and protocols used in this study.

Post Groups Preparation

In the CP1, CP2, and CP6 groups, the Unicore Post and Drill System was used, which consisted of a radiopaque fiber post of conical design and a drill for the preparation of the post space. The post is characterized by prestressed quartz fibers encased in a bondable resin matrix that creates a monoblock

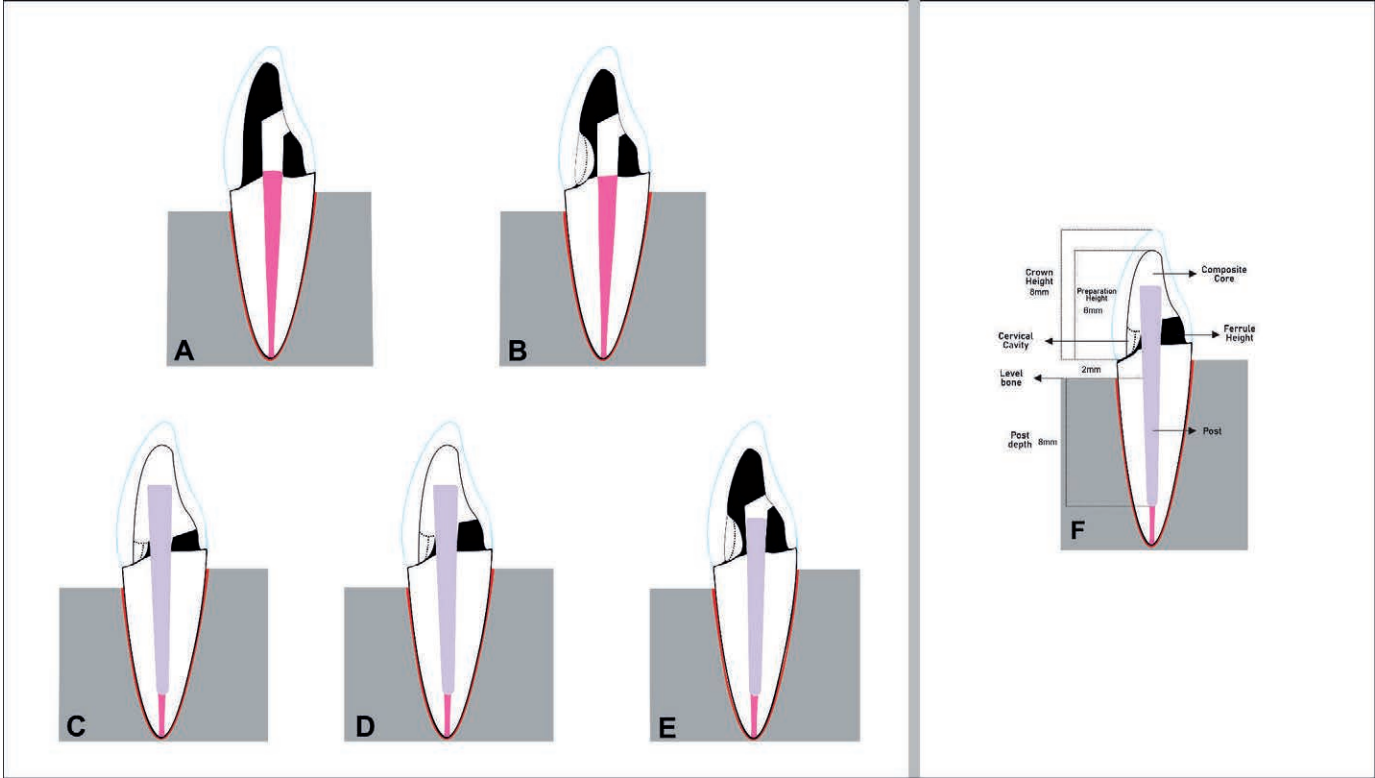


Figure 1. The experimental groups showing the cervical cavity, preparation of the ferrule, fiber post-and-core and crown. (A): specimens of the control group (CG) 6-mm ferrule height, without cervical cavity and no post. (B): specimens of Group (CO) 6-mm ferrule height and only with cervical cavity. (C): specimens of Group (CP1) 1-mm-height ferrule with cervical cavity and fiber post. (D): specimens of Group (CP2) 2-mm-height ferrule with cervical cavity and fiber post. (E): specimens of Group (CP6) 6-mm-height ferrule with cervical cavity and fiber post (F): schematic design illustrating the dimensions of the preparation, post, and crown.

Table 1: Materials, Composition, and Protocols Used in This Study		
Material	Composition	Protocol Used in This Study
Fiber post	Quartz fibers Resin matrix	Manufacturer's instructions
Multilink N	Monomer matrix of dimethacrylate and HEMA Inorganic fillers: Barium glass, ytterbium and spheroid mixed oxide	Manufacturer's instructions
Monobond Plus Primer A and B	Silane methacrylate, phosphoric methacrylate, sulfide methacrylate Primer A: Water, initiators, sulfonate amine Primer B: HEMA, phosphonic acid acrylate, methacrylate modified polyacrylic acid, stabilizers	
Single bond universal	Bis-GMA, HEMA, dimethacrylates, ethanol,water, polyacrylic and polytaconic acids	Manufacturer's instructions and cured for 40 s
Composite resin Z350 Filtek	Bis-GMA, TEGDMA, Bis-EMA, UDMA, Zirconia/silica filler	Manufacturer's instructions
Nickel-cromo alloy VeraBond II	Ni 76.5%, Cr 11.5%, Mo 3.5%, Nb, Al, Si, Ti	Manufacturer's instructions
Glass ionomer cement Fuji Plus	Ingredient—Powder: Aluminosilicate 100% Ingredient—Liquid: Distilled water 35%, polyacrylic acid 45%, HEMA 15%, dimethacrylate 5%	Manufacturer's instructions
Self-curing acrylic resin for tooth mounting	2-Propenoic acid, 2-methyl-, ethyl ester, homopolymer, benzoyl peroxide	Manufacturer's instructions
Abbreviations: Bis-GMA, bisphenol A diglycidyl methacrylate; Bis-EMA, bisphenol A diglycidyl methacrylate ethoxylated; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; HEMA, 2-Hydroxyethyl metacrylate.		

when the core material is placed. The post selected in the study was size 3 (blue), with an apical diameter of 1.17 mm and coronal diameter of 1.56 mm (UniCore UP, Ultradent Products). For the post preparation in CP1, a Peeso No. 2 reamer (Union Broach Co, York, PA, USA) was used to remove the gutta-percha to a depth of 9 mm measured with a stop from the remaining coronal structure. In the groups, CP2 (2 mm) and CP6 (6 mm), the stop was placed at 10 and 14 mm, respectively. As a result, the length of the post space was consistent at 8 mm for all the groups. The post space was then gradually widened using a Peeso reamer No. 3 and 4 (Union Broach Co). For the final preparation of the post space, a blue helicoidal bur size 3 (UniCore UP 7123, Ultradent Products) was used to a depth of 8 mm, which more precisely defined the shape of the post space to the matching post.

The posts were cemented with a self-curing resin cement (Multilink N, Ivoclar Vivadent), following the manufacturer's instructions. The surface of the post was cleaned with an alcohol-saturated cotton swab, then Monobond N (Ivoclar Vivadent) was brushed on the surface of the post, allowing it to react for 60 seconds before air dispersing with dry clean air. The post space was irrigated with distilled water and dried with paper points (Coltene Whaledent, Altstätten, Switzerland). Primer A and B (Multilink N, Ivoclar Vivadent) were mixed with a microbrush and introduced into the post space. After 15 seconds, excess material was removed with paper points (Coltene). The cement mix was coated on the fiber post, and the post was seated using light repetitive torsional movements to reduce hydraulic pressure and prevent air entrapment. Digital pressure consistent with clinical practice was applied followed by visible light irradiation for 20 seconds (VALO Cordless, Ultradent Products). The LED light activation produces a higher adhesion strength of the resin cement to the intraradicular dentin in the coronal region.⁴² The remaining coronal walls of groups CP1, CP2, and CP6, as well as the root canal access and cervical cavity of the groups CG and CO were etched with 37% phosphoric acid (Prime Dental) for 15 seconds, rinsed with a triple air-water syringe and then gently dried. With a microbrush, a dentin adhesive was applied (Single Bond Universal, 3M Oral Care, Seefeld, Germany), which was light cured for 40 seconds (VALO Cordless; Ultradent Products) because when a long photoactivation time is used in adhesive systems, the level of polymerization increases.^{43,44} The VALO light is high intensity and had an output of 1000 mW/cm².

Then, the cervical cavity and core were built in increments with composite resin (Z350 Filtex, 3M Oral Care), each increment light cured for 20 seconds.

With a diamond high speed bur (no. 5856.31.018; Brasseler), under copious irrigation, the preparations were finalized to meet the following parameters: a height of 6 mm, a rounded shoulder finish line extending apically to the cervical cavity and in the other areas at the CEJ with a reduction of 1.0 mm on the facial, 1.0 mm interproximally and lingually, and an incisal reduction of 2 mm.

Fabrication and Cementation Crowns

One specimen was coated with 1 layer of die spacer (Die Spacer, Keystone Industries, Gibbstown, NJ, USA), and a complete contour wax pattern was fabricated. This pattern measured 8-mm high, with a circumferential thickness of, approximately, 1–1.5 mm. A rectangular-shaped stop with a central concavity was added 2-mm apical to the incisal edge on the lingual surface. This stop facilitated loading during the fracture resistance tests. An impression in vinyl polysiloxane (Putty Normal Set, Elite HD, Zhermack, Badia, Polesine, Italy) was made of the wax pattern, which served as a mold for replicas of all other samples. Final adjustment of the wax replicas was carried out for each individual sample to obtain good marginal adaptation.

The wax patterns were cast in nickel–chrome alloy (VeraBond II, AalbaDent, Inc, Fairfield, CA, USA). After finishing, a resin-reinforced glass ionomer cement was mixed, according to the manufacturer's instructions (Fuji Plus, GC, Alsip, IL, USA), and used for cementation. The castings were held under finger pressure until initial set had occurred.

Fracture Load Test

After 24 hour storage in water at room temperature, the specimens were thermocycled in distilled water (6000 cycles; 5°C/55°C, 2 minutes each cycle) to mimic clinical conditions.⁴⁵ The roots were embedded in self-curing translucent acrylic resin that had a curing time of 5–8 minutes (SamplKwich Kit, Buehler, Lake Bluff, IL, USA), leaving 2 mm of the root surface exposed while following the contour of the CEJ to mimic bone support.

Each sample was subjected to a fracture resistance test using a universal testing machine (5567, Instron, Norwood, MA, USA). A compression force was applied to the stop on the lingual surface of the crowns by means of a spherical tip with a 3-mm

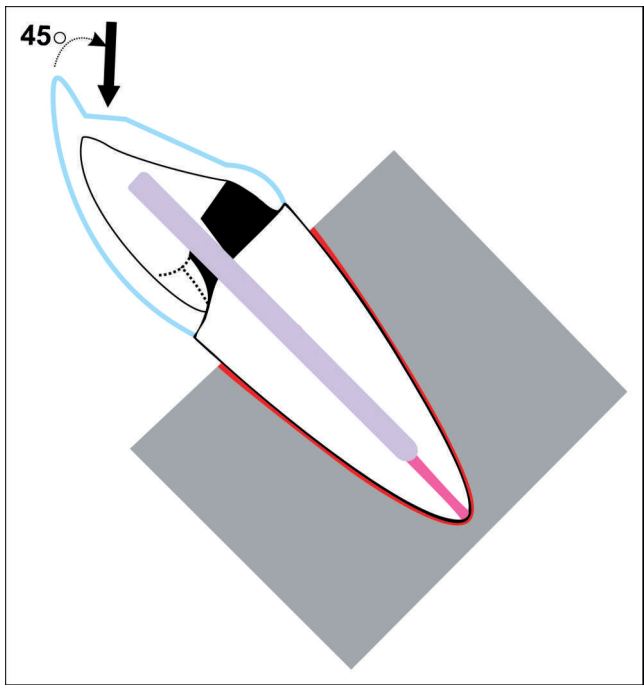


Figure 2. Schematic representation of the tooth under static load.

diameter. The force was directed at an angle of 45° to the longitudinal axis of the tooth at a crosshead speed of 0.5 mm/min, until fracture. This angle was based on the natural occlusal forces on maxillary incisors in a Class I occlusion⁴¹ (Figure 2).

Failure Mode Analysis

The mode of failure was evaluated by embedding the specimens in self-curing acrylic resin (SamplKwich, Buehler). Two sections were made in a bucco-lingual direction, using a low-speed diamond blade under water irrigation (IsoMet 5000, Buehler). They were photographed with a digital microscope (VHX-1000, Keyence, Osaka, Japan) at $\times 20\times$ magnification. The failure mode, based on Fokkinga and others⁴⁶, was characterized as follows: complete decementation of the post-and-core and crown; partial decementation of the post-and-core and/or crown; fracture of resin composite foundation–coronal dentin; dislodgement of lingual cervical margin of the crown; root fracture trajectory (oblique or horizontal); fracture propagation (subosseous, supraosseous); favorable failure (failure allows restoration of the tooth); and unfavorable failure (failure does not allow predictable restoration).

Data Analysis

The data were analyzed by analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK)

Table 2: Fracture Load (N) of Different Groups

	Group	Mean±SD
CG	6-mm ferrule height, no cervical cavity and no post	667±119
CO	6-mm ferrule height with cervical cavity	668±119
CP1	1-mm ferrule height with cervical cavity and post	531±125*
CP2	2-mm ferrule height with cervical cavity and post	608±192
CP6	6-mm ferrule with height cervical cavity and post	769±175*

*Statistically significant differences ($p<0.05$) between groups.

multiple comparison statistical test ($\alpha=0.05$) (Sigma-Plot 14; Sytat Software, San Jose, CA, USA).

RESULTS

The fracture resistance and standard deviation data are presented in Table 2. The CP6 group presented the highest fracture load resistance (769±175 N), the CP1 group being the lowest (531±125 N). An ANOVA analysis showed a statistically significant difference between these two groups ($p<0.05$). With respect to the groups with 6-mm high dentinal axial walls, without and with a cervical cavity, fracture resistance was similar CG (667±119 N) and CO (668±119 N). In the CP6 group with cervical cavity and 6-mm-height remaining walls, the placement of the post increased fracture resistance (769±175 N), although this was not statistically significant in relation to the CG and CO groups. In the CP2 group with 2-mm-high remaining walls with cervical cavity and with a post, the fracture resistance was 608 ± 192 N, close to the CG and CO group values.

A partial post-core and/or crown decementation was identified in all CG and CP6 specimens, in nine CP1 and CP2 specimens, and in three CO specimens. The dislodgement of the cervical margin on the lingual of the crowns occurred in seven CG specimens and in all CO, CP1, CP2, and CP6 specimens. Fracture of the composite resin–dentin core occurred in seven CP1 specimens, in five CP2 specimens, and in four specimens of groups CG, CO, and CP6. Horizontal and oblique fractures occurred in few specimens, mostly in CP6. The location and trajectory were more frequently at the level of the cervical and middle-thirds of the root, and at the apical limit of the post. The propagation of subosseous cracks occurred in approximately half of all the specimens, slightly below the margin of the acrylic resin that simulated the level of the bone crest. Failures were considered favorable when there was sufficient

Table 3: Failure Modes					
Failure Modes ^a	Experimental Groups				
	CG	CO	CP1	CP2	CP6
Complete decementation of postcore and crown				1	
Partial decementation of postcore and/or crown	10	3	9	9	10
Fracture composite resin foundation–coronal dentin	4	4	7	5	4
Dislodgement of lingual cervical margin crown	7	10	10	10	10
Oblique fracture		2	1		3
Horizontal fracture	5	4		4	4
Propagation fracture subosseous	4	6	2	4	7
Propagation fracture supraosseous	6	1	3		3
Failure favorable/unfavorable total	6/4	4/6	8/2	6/4	3/7

^a Based on Fokkinga and others,⁴⁶ and Santos Pantaleón and others.²⁸

remaining dentin to allow for a preparation of a new cervical finish line. While unfavorable failures compromised the integrity of the tooth, making it unrestorable (Table 3). Figure 3 shows specimens with failures.

DISCUSSION

The reason for this laboratory research was based on published arguments by Jotkowitz & Samet.³² The authors stated: “The evidence relating to restoring the endodontic treated tooth with extensive destruction is

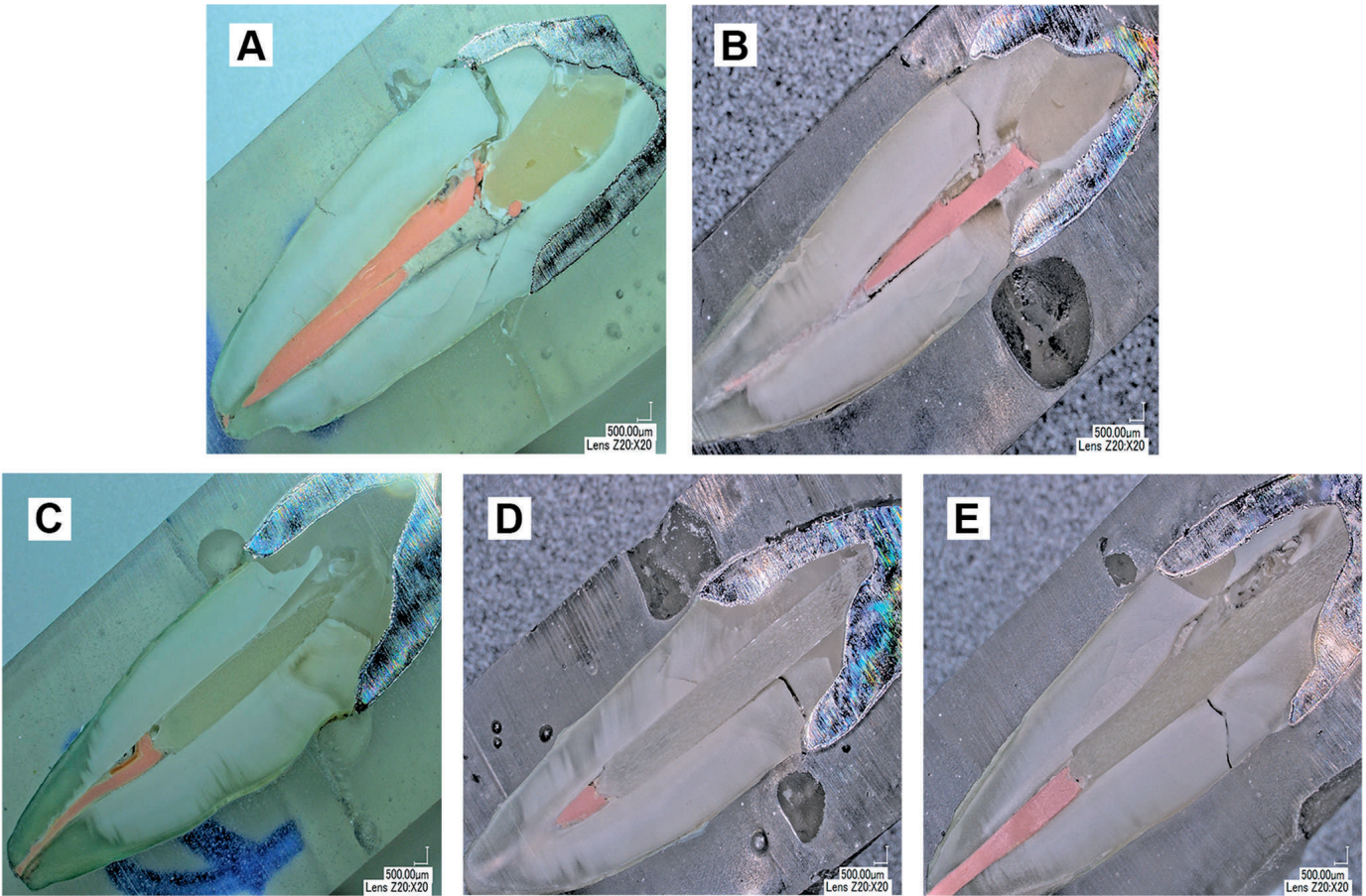


Figure 3. Representative samples of failures observed using digital microscopy. A: Group CG specimen with coronal dentin fracture and dislodgement of lingual cervical crown margin; B, Group CO lingual supraosseous fracture; C, Group CP1 dislodgement of lingual cervical crown margin and core displacement; D, Group CP2 horizontal fracture of coronal dentin; E, Group CP6 oblique root fracture.

deficient.” To the best of our knowledge, this was the first study to investigate the effect of facial cervical lesions on fracture resistance in the endodontically treated maxillary central incisor restored with a complete crown. While this does not occur frequently,³⁹ it is still of clinical importance. The fracture resistance of the CO group (668 N) with dentin walls 6 mm in height with a cervical cavity and without a post was similar to the CG group (667 N) that had a ferrule of 6 mm in height without a cervical cavity and post. While in the CP6 group (769 N) with 6-mm-high walls and a cervical cavity and a post, the fracture resistance increased, although there was no statistically significant difference. According to these results, the null hypothesis that placement of a fiber post would not affect the fracture resistance of maxillary central incisors restored with a complete crown and a facial cervical lesion was rejected.

The samples that had endodontic treatment only (CG) recorded no difference in fracture load compared to the teeth that had endodontic treatment and a cervical lesion (CO). This may be due to a combination of facts, such as the large amount of the remaining coronal structure, height of dentinal walls, and the protective effect of the complete crown, all contributing to a better distribution of stress.²⁰ This suggests that the loss of dentin caused by a cervical lesion did not affect the fracture resistance between these two groups.

Groups CP6 and CO had similar-height walls in dentin and cervical lesions. It was observed that the placement of a fiber post created a positive effect, because it increased the fracture resistance CP6 (769 N) compared to the CO group (668 N) without a post, albeit it was not statistically significant. This finding suggests that a fiber post and resin cement contribute to the strengthening of the tooth, which is in accordance with other studies.^{7,9} Posts also play a role in absorbing energy⁴⁷ and reduce stress on the tooth due to the distribution of the occlusal load within the root dentin.⁴⁸ This is consistent with the previous reports showing that a post increases fracture resistance when compared to a CG without post with the same remaining wall height.^{17,41,49} In addition, the reduction of tooth structure due to preparation and endodontic treatment causes an increase in the deflection of the tooth, exposing it to more stress.⁴⁸ In that sense, the fiber post increases the load values and decreases the deflection of the compromised tooth structure.⁵⁰ Another factor that could explain the findings mentioned above is the location of the defect in the tooth. A study has shown that there was no difference in fracture resistance

compared to indirect restoration with a ceramic crown when the defect is a Class III (mesial and distal restored with direct composite resin).³ This indicates that whether or not a fiber post is present, the fracture resistance is not affected.^{3,12} On the other hand, if a cervical cavity is present in the facial, a post exerts a positive effect, as was demonstrated by an increase in fracture resistance.³⁹ This difference between cervical cavity and class III defects can be explained, as the endodontically restored tooth is subject to more stress in the cervical region,^{16,19,31} while the presence of a post reduces stress.¹⁹ According to a finite element study, a decrease in root dentin thickness in the cervical area contributes to high levels of tension within the root canal.¹⁵

Regarding the type of coronal restoration, it has been suggested that the mechanical properties of the crown affects the resistance to fracture,⁷ and the level of stress and strain along the dentin–cement–post interface.⁹ Although the use of ceramic crowns has increased considerably in the anterior region due to its esthetic qualities and advantage to be adhesively cemented,²⁶ metal–ceramic crowns continue to be indicated in clinical practice.⁷ The aim of the present study was to compare the influence of the facial cervical cavity and a fiber post on the resistance to fracture. For this purpose, the use of a metal crown is preferable, because it is more suitable for evaluating the ferrule effect, as it allows stresses to concentrate more on the remaining coronal dentin, according to a finite element study.¹⁶

The microscopic evaluation of the failure mode of the sections of the selected specimens allowed precise detection of the type of failure. The CP6 group presented a greater number of catastrophic failures, identifying seven specimens, in relation to CO and CG with 6 and 4 catastrophic failures, respectively. This result can be attributed to the presence of a fiber post, as it serves as a distributor of stress and load to the core and crown within the root canal.^{9,19} In the absence of a post, the concentration of stress in the canal was insignificant.¹⁹ That could explain the horizontal and oblique root fractures located in the middle-third and at the apical limit of the post in the CP6 group. Subosseous propagation of crack also occurred in the majority of the CP6 group and was characterized to a greater extent on the surface of the dentin. The results of the present study show 70% nonrepairable failures in CP6, while another study showed 100% and has questioned the use of a fiber post.²

The fracture resistance of CP1 with a cervical cavity, a post and 1-mm-high ferrule (531 N) was

inferior compared to CP6 that had a cervical cavity, a post and 6-mm-high ferrule (769 N); the difference was statistically significant ($p < 0.05$). This showed that tooth resistance is directly related to the amount of remaining dentin, which corroborates the findings of other studies.^{17,27} However, the CP1 group had an unfavorable failure in only two specimens compared to the CP6 group with seven specimens. Due to the minimal coronal structure, high stresses are concentrated in the cervical area,⁶ which causes fracture at the level of the resin composite–dentin foundation, which was characterized as a favorable failure. With an increase in coronal structure, stresses are transmitted to the apical area,^{6,22} causing a greater number of root fractures, as evidenced in CP6.

Despite the intrinsic limitations of a static load, it is a frequently applied methodology in laboratory studies to evaluate the ability to resist fracture, because it is an efficient method due to its low cost while being less time consuming. Reproducing exact oral conditions in laboratory studies is not possible. However, in this study, the specimens were subjected to thermocycling to mimic clinical conditions and simulate aging of the restoration before a load was applied. This study observed that facial cervical lesions affect the configuration of the ferrule and influence fracture resistance in maxillary central incisors under static load. More studies are needed to assess the significance of the thickness of remaining dentin in the cervical lesion after the ferrule preparation. Furthermore, the performance of different types of fiber posts, cementation protocols, and luting agents and the composition of crown materials need to be examined after applying dynamic load testing.

CONCLUSIONS

Within the limitations of this study it is suggested that, when an endodontically treated maxillary central incisor that has a cervical lesion needs to be restored with a complete crown, a fiber post is cemented to improve fracture resistance.

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

Regulatory Statement

The author represents that the study was performed in compliance with author's institution's appropriate policies related to the use of animal and/or human subjects and

human-derived material and the policies of National Council of Bioethics in Health of the Dominican Republic CONABIOS. The approval code issued for this study is 16/1/2015.

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Demineralization Inhibition by Two Calcium-releasing Restorative Materials

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Clinical relevance

Calcium-releasing materials may be able to inhibit demineralization at the cementum margins of direct restorations better than resin composites. However, resin-modified glass ionomers (RMGIs) seem to provide a higher amount of protection from demineralization.

SUMMARY

Objective: To compare the ability of two calcium-releasing restorative materials to inhibit root dentin demineralization in an artificial caries model.

Methods and Materials: Preparations were made at the cementum–enamel junction of extracted human molars (40, n=10/material) and restored with two calcium-releasing materials (Experimental composite, Pulpdent Cor-

poration and Cention N, Ivoclar Vivadent), a resin composite (Filtek Supreme Ultra, 3M Oral Care), and a resin-modified glass ionomer (RMGI) (Fuji II LC, GC). All materials (other than the RMGI) were used with an adhesive (Scotchbond Universal Adhesive, 3M Oral Care) in the self-etch mode, which was light cured for 10 seconds. All restorative materials were light cured in 2-mm increments for 20 seconds and then finished with a polishing disc. Teeth were incubated (37°C) for 24 hours in water. An acid-resistant varnish was painted onto the teeth around the restoration, leaving a 2-mm border of uncovered tooth. A demineralization solution composed of 0.1 M lactic acid, 3 mM $\text{Ca}_3(\text{PO}_4)_2$, 0.1% thymol, and NaOH (to adjust pH=4.5), and a remineralization solution composed of 1.5 mM Ca, 0.9 mM P, and 20 mM Tris(hydroxymethyl)–aminomethane (pH=7.0) were prepared. Specimens were placed in the demineralization solution for 4 hours, followed by the remineralization solution for 20 hours and cycled daily for 30 days. The specimens were embedded, sectioned into 100- μm sections, and the interface between the restorative material and root dentin was viewed with polarized light microscopy. A line was drawn parallel with the zone of deminer-

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alization for each tooth. The area of “inhibition” (defined as the area external to the line) or “wall lesion” (defined as the area internal to the line) was measured with image evaluation software. Areas of inhibition were measured as positive values, and areas of wall lesions were measured as negative areas.

Results: A one-way analysis of variance (ANOVA) found significant differences between materials for “inhibition/wall lesion” areas in root dentin ($p < 0.001$). Tukey post hoc analysis ranked materials (μm^2 , mean $\pm$ SD): Fuji II LC (5412 ± 2754) > Cention N (2768 ± 1576) and experimental composite (1484 ± 1585) > Filtek Supreme Ultra (-1119 ± 1029).

Conclusion: The experimental composite and Cention N materials (used with an adhesive) showed net areas of inhibition greater than a reference resin composite, albeit at a lower level than a reference RMGI material (used with no adhesive).

INTRODUCTION

In July 2018, a group of 50 key opinion leaders met to develop a consensus statement on the definition of a bioactive restorative dental material.¹ From that consensus meeting, a bioactive restorative material was defined as one that would restore missing tooth structure and additionally “stimulate or direct specific cellular or tissue responses or control interactions with microbiological species”.¹ One example of a bioactive effect was described as having “components that dissolve and can be identified with normal physiologic species involved in a biological process.” During the process of secondary caries, hydroxyapatite is detached from the surface of the tooth surrounding a dental restoration. A restoration that can release the ions present in the mineral content of the tooth would, in theory, be able to assist the process of remineralization in the tooth structure surrounding the restoration and decrease secondary caries.² As calcium and fluoride are components of hydroxyapatite or fluorapatite in tooth structure, materials that release fluoride (such as glass ionomers) and those that release calcium could both be considered bioactive. The recent trend, however, has been to identify calcium-releasing materials as bioactive, since this is the major component of hydroxyapatite.

Glass ionomer materials release fluoride in both an initial burst (first 24 hours) when glass particles react with polyalkenoic acid during the setting reaction and

a prolonged long-term release when glass dissolves in the acidified water of the hydrogel matrix.^{3,4} The fluoride release from glass ionomer materials plateaus after 10-20 days,⁵ and continued low-level fluoride release has been reported for up to 3 years.⁶ A similar release profile has been reported for resin-modified glass ionomer (RMGI) materials.⁷ Polyacid-modified composites (also called compomers) do not have a burst release of fluoride; however, they have a constant fluoride release at lower level than glass ionomer or RMGI materials.^{8,9} Fluoride release is increased from restorative materials when they are placed in an acidic solution, presumably due to dissociation of the material.^{10,11} Application of fluoride-containing solutions may provide a so-called recharge of restorative materials that may allow sustained fluoride release by washout of fluoride retained on the surface of a material or from ion-containing solute within pores of the material.¹² As glass ionomer materials are more permeable and have higher water sorption than resin-based materials, they act as a better reservoir for fluoride.¹²⁻¹⁵

Fluoride released from dental materials can be incorporated structurally into surrounding tooth structure (fluorapatite) or precipitate onto the tooth surface as a calcium fluoride layer.¹² Structural incorporation of fluoride in teeth produces larger apatite crystals, which increases resistance to carious lesion formation.¹⁶ A deposited fluoride layer may facilitate reprecipitation of minerals, also helping to prevent mineral ion loss caused by the caries process.¹⁷ The amount of fluoride uptake from ion-releasing restorative materials is higher in dentin than enamel.¹⁸ Several laboratory studies have demonstrated the ability of fluoride-releasing materials to inhibit demineralization of surrounding enamel or root dentin.¹⁹⁻²⁸ A clinical study has also shown less demineralization around extracted primary teeth restored with glass ionomer than with amalgam.²⁹ Clinical trials have reported a higher incidence of secondary caries around resin composite and amalgam restorations than fluoride-releasing materials, particularly in patients who were non-compliant with extrinsic fluoride application.³⁰⁻³³ The clinical benefit of fluoride-releasing materials is not evident in all clinical trials. A systematic review of 28 clinical trials reported that there was insufficient evidence to support a treatment effect of inhibition of secondary caries by glass ionomer materials. This review reported that there was an equal number of studies with an incidence of caries in the glass ionomer group as those with an incidence of caries in the control group. The

Table 1: Materials Used in This Study			
Material	Manufacturer	Matrix	Filler
Filtek Supreme Ultra	3M Oral Care	Methacrylate resins	Silica, zirconia
Experimental premarket version of Activa Presto	Pulpdent Corporation	Methacrylate resins with added polyacid monomers	Fluoro Aluminosilicate glass, silica
Cention N	Ivoclar Vivadent	Methacrylate resins	Calcium fluorosilicate glass, calcium barium aluminum fluorosilicate glass, barium aluminum silicate glass
Fuji II LC	GC	Polyacrylic acid with added methacrylate functional groups	Fluoro Aluminosilicate glass

magnitude of the difference in incidence of caries for either material was not analyzed.³⁴

Several calcium-releasing restorative materials are commercially available with claims of bioactivity. Some of the bioactive restorative materials currently on the market, such as Activa BioACTIVE-Restorative (Pulpdent Corp, Boston, MA, USA), could be considered a compomer, as its polymer matrix is predominantly composed of a dual-cured resin methacrylate with added polyacid monomers. A recent study identified the filler as fluoroaluminasilicate glass.³⁵ Previous studies have reported small amounts of calcium released from Activa BioACTIVE-Restorative.^{35,36} A recent study identified a spherical calcium-containing filler particle within the material that may be responsible for the release.³⁶ A new, modified, single syringe delivery system is now available on the market (Activa Presto, Pulpdent). An experimental premarket version of this material, which can also be considered a compomer, was tested in the present study. Another commercially available calcium-releasing restorative material, Cention N (Ivoclar Vivadent, Lichtenstein, Schaan), which contains methacrylate monomers as well as calcium fluorosilicate glass fillers, was also tested in the present study.³⁵ Cention N has been shown to inhibit caries formation at restoration margins *in vitro*.³⁷

The purpose of the present study was to determine if two calcium-releasing materials (an experimental material and Cention N) could inhibit demineralization of surrounding root dentin relative to reference RMGI (positive control) and resin composite (negative control) materials. The study methodology was cyclic remineralization and demineralization of restorations placed in extracted human teeth. The null hypothesis was that there would be no difference in the area of demineralization inhibition provided by the two calcium-releasing materials relative to the reference resin composite or RMGI materials.

METHODS AND MATERIALS

Forty freshly extracted human molars (mixture of maxillary and mandibular, and 1st-3rd molars) from unidentified donors were collected from the Oral and Maxillofacial Surgery department. Caries-free molars with relatively flat buccal surfaces were selected. Prior to restoration, the teeth were immersed in distilled water for up to 6 months and randomized into four groups of 10 specimens each. Approximately 4 mm × 3 mm (±0.2 mm) box preparations (2-mm deep) were prepared on the buccal surfaces of the human molars with an enamel occlusal margin, while the gingival margin was placed in root dentin. All preparations were performed with a #557 carbide bur in a high-speed handpiece with copious water irrigation.

One resin composite (Filtek Supreme Ultra, 3M Oral Care, St Paul, MN, USA), two calcium-releasing materials (Experimental material, Pulpdent; Cention N, Ivoclar Vivadent), and one RMGI material (Fuji II LC, GC America, Alsip, IL, USA) were used to fill the prepared box (Table 1). For the Filtek Supreme Ultra, Cention N and experimental material groups, an adhesive (Scotchbond Universal, 3M Oral Care) used in the self-etch mode was applied with 20 seconds of agitation followed by solvent evaporation with 5 seconds of gentle air. The adhesive was then light cured with an LED curing light (Elipar S10, 3M Oral Care, output > 1000mW/cm²) for 10 seconds. The restorative materials were then placed into the preparations and light cured for 20 seconds using an LED curing light (Elipar S10). After finishing and polishing, the restorations were examined using a digital microscope (VHX-600, Keyence, Osaka, Japan) under 20× magnification to ensure an effective marginal seal was present, and there was no excess material present over the restoration margin. Any resin composite flash present over the restoration margin was removed with a polishing disk (medium Sof-Lex disc, 3M Oral Care).

Afterwards, the teeth were incubated for 24 hours in distilled water at 37°C to allow complete polymerization of all materials. The surface of the teeth were dried with a paper towel. Acid-resistant varnish with different colors was painted onto the teeth for different groups, leaving a window including the restoration and 2 mm of tooth structure uncoated surrounding the restoration.

An artificial caries model was used based on a previously published protocol.^{27,38} A 500 mL demineralization solution composed of 3.7 mL lactic acid (0.1 M), 0.111 g CaCl₂ (2 mM Ca) and 0.136 g KH₂PO₄ (2 mM PO₄), 0.5 g thymol (0.1% to prevent bacterial growth), and NaOH (to adjust pH=4.5) was prepared. A 500 mL remineralization solution composed of 0.083 g CaCl₂ (1.5 mM Ca), 0.061 g KH₂PO₄ (0.9 mM Ca), 0.121 g *Tris*-(hydroxymethyl) aminomethane (20 mM, pH=7.0), and 0.5 g thymol (0.1% to prevent bacterial growth) was prepared. The specimens were immersed in the demineralization solution for 4 hours, followed by the remineralization solution for 20 hours and cycled daily. A 5-minute wash in running deionized water was performed between each cycle. To ensure that the solution was agitated through the experiment, a magnetic stirrer was utilized in both the demineralization and remineralization solutions.

After 1 month, the specimens were embedded into polymethyl methacrylate (Ortho-Jet, Lang Dental Manufacturing Co, Wheeling, IL, USA) and sectioned into approximately 150 µm sections using a low-speed sectioning saw (IsoMet, Buehler, Lake Bluff, IL, USA). Two cuts were made through the buccal-lingual plane of the tooth through the mesial-distal center of the restoration to obtain the 150-µm section. The sections were then hand polished with 600 grit silicon carbide paper to achieve a final thickness of 100 µm ± 20 µm. Any sections with artifacts (flash over the margin, voids at the margin) were discarded, and an additional section was taken from the specimen. A small number of specimens separated at the tooth-restoration interface during polishing.

Sections were viewed with polarized light microscopy at 10× (VHX-600, Keyence). The zone of demineralization was defined as the area in which a visual detection in the appearance of the root dentin could be viewed. The zone of demineralization started at the external surface of the tooth and progressed in a pulpal direction. For some specimens, an “area of inhibition” was observed adjacent to the restoration in which the zone of demineralization did not progress as deep in the pulpal

direction. For other specimens, a “wall lesion” was formed in which the zone of demineralization progressed deeper in the pulpal direction at the area adjacent to the restoration. A line demarking the front line of the zone of demineralization was defined for each tooth (Figure 1, through point A and point B to point C). An “area of inhibition” was defined as any area of unaltered root dentin adjacent to the restorative material and shallower (away from pulp) than the line (Figure 1, area between points B, C, and D). A “wall lesion” was defined as any area of demineralized root dentin that was adjacent to the restorative material and deeper (towards pulp) than the line (Figure 1, area between points B, C, and E). These areas were hand traced with internal image evaluation software and measured. Areas of inhibition were recorded as positive values, and areas of wall lesions were recorded as negative values.

Data were compared with one-way analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) analysis. The significance level was set at $\alpha=0.05$. All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

All specimens in the Filtek Supreme Ultra group (negative control) produced wall lesions; whereas, all specimens in the Fuji II LC, experimental composite, and Cention N groups produced “areas of inhibition.” The mean and standard deviation of the “areas of inhibition” and “wall lesions” were as follows: Fuji II LC (5412±2754), Cention N (2768±1576), experimental composite (1484±1585), and Filtek Supreme Ultra (−1119±1029). The measurements of each specimen are listed in Table 2. Negative or positive values indicate from which side of the front line of demineralization the area is measured. The negative values for Filtek Supreme Ultra denote that the wall lesions were located deeper (towards pulp) than the front line of the zone of demineralization. The one-way ANOVA determined that there were significant differences between materials ($p<0.01$). The Tukey post hoc analysis determined that the experimental composite and Cention N groups had no statistically significant difference but produced smaller zones of inhibition than the Fuji II LC group. Representative specimens from each group are shown in Figures 2-5.

DISCUSSION

In this study, there was significantly more demineralization inhibition adjacent to the Cention N and the experimental material than the reference resin composite but less than that adjacent to the RMGI

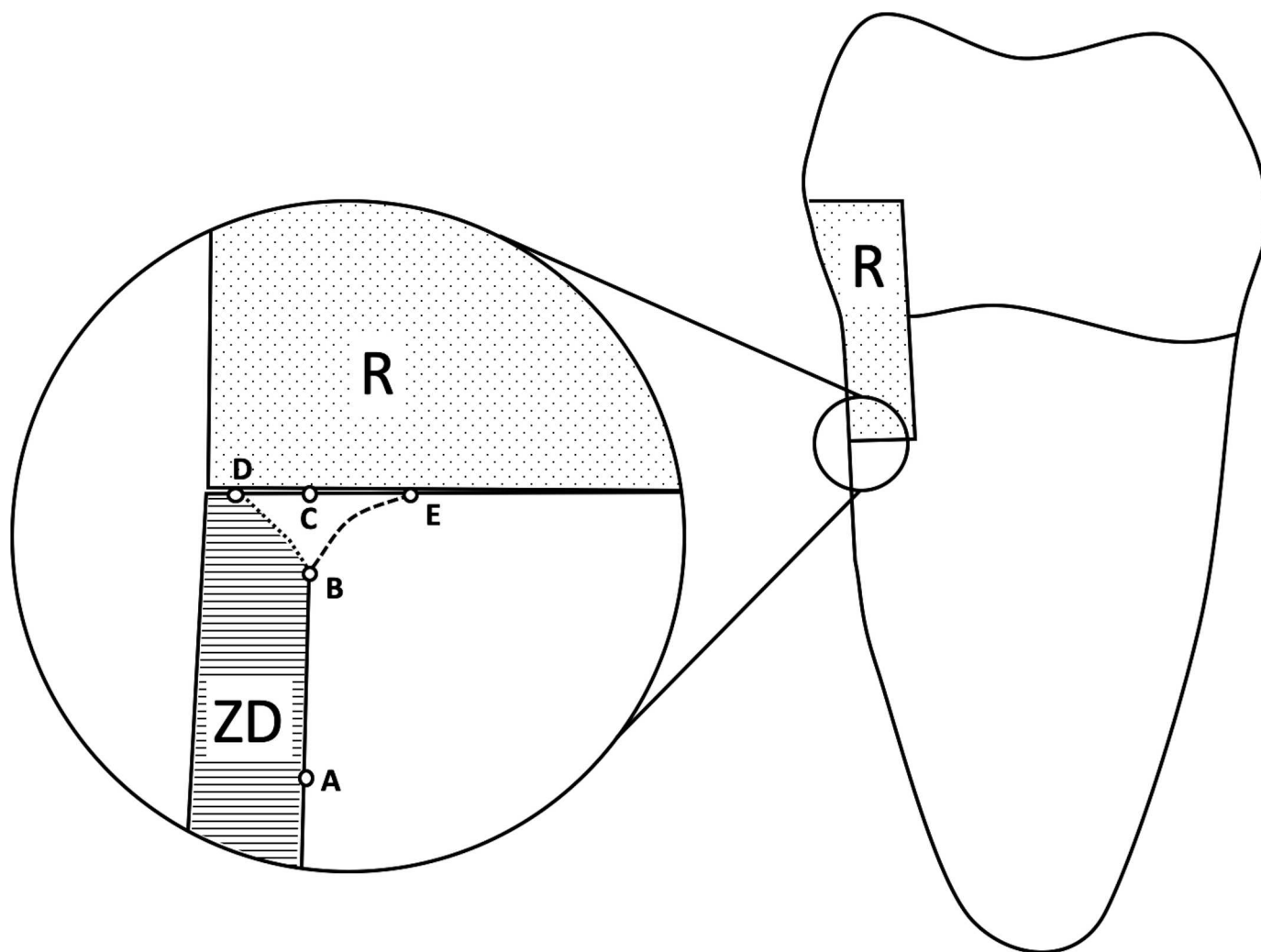


Figure 1. Schematic of interface between restoration (R) and root dentin. Zone of demineralization (ZD) defined as area in which a visual detection in the appearance could be viewed. Area of inhibition defined by nondemineralized root dentin bounded by points B, C, and D. Wall lesion defined as demineralized root dentin bounded by points B, C, and E.

material. Therefore, we reject the null hypothesis. The ability of this test to discriminate between different materials demonstrates its utility.

Previous studies have shown that fluoride-releasing restorative materials can inhibit demineralization around both enamel and dentin. One previous study reported that materials ranked in their protective ability (from the most to the least): glass ionomer, RMGI, giomer, compomer, and resin composite.²⁰ Several studies have confirmed that glass ionomers and RMGI materials provide more caries inhibition than resin composites.^{19-22,26-28} One study reported that a compomer material provided similar protection as glass ionomer and/or RMGI materials²¹; whereas, others reported that compomers provided less protection.^{20,22,26}

Both Cention N and the experimental material could be considered to be similar to compomers, as they contain methacrylate functional groups and fluoride-releasing fillers. A previous study reported that Cention N releases more fluoride than Fuji II LC.³⁹ A previous version of Activa BioACTIVE-Restorative showed very little fluoride release, less than Cention N.³⁵ The differentiating characteristic of Cention N and the experimental materials from glass ionomers or RMGIs is that they are formulated with calcium-containing components; whereas, the RMGI used in this study does not release calcium. Cention N releases significantly more calcium than a previous version of Activa BioACTIVE-Restorative.³⁵ Despite the additional calcium release, both Cention N and the experimental material showed less demineralization inhibition than that provided by

Table 2: Areas of "Inhibition" (Positive Values) or "Wall Lesions" (Negative Values) in Root Dentin Adjacent to Different Restorative Materials

	Fuji II LC	Cention N	Experimental Composite	Filtek Supreme Ultra
1	7557	2803	1536	-1495
2	3426	0	275	-1510
3	9410	2919	1256	-812
4	3116	0	-93	576
5	2055	3905	4809	-756
6	2612	3013	3506	-2836
7	9231	3853	112	-134
8	6892	4747	1998	-337
9	3890	3497	441	-1595
10	5928	2944	998	-2289
Average	5412 a	2768 b	1484 b	-1119 c
Standard Deviation	2754	1576	1585	1029

*Values with different letters are significantly different.

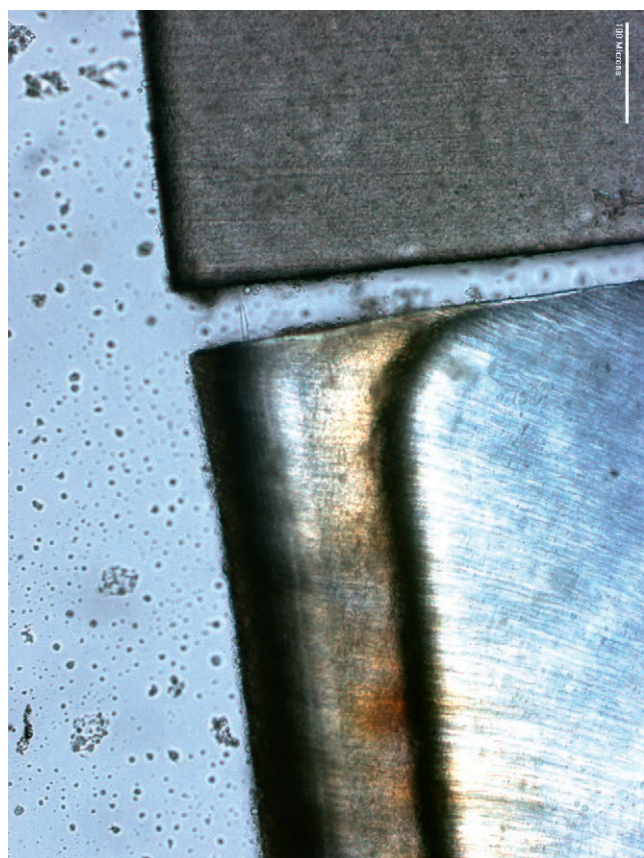


Figure 2. Representative specimen showing a wall lesion in root dentin (bottom) adjacent to a Filtek Supreme (resin composite) restoration (top). Note that the gap between the restorative material and the tooth occurred in several specimens during the process of cross-sectioning the teeth.



Figure 3. Representative specimen showing an area of inhibition in root dentin (bottom) adjacent to an experimental calcium-releasing restoration (top).

Fuji II LC—a RMGI material. Our study confirms previous findings by Donly, who also reported that Cention N produced less protection than a RMGI material.³⁷ Figures 4 and 5 demonstrate that both Cention N and Fuji II LC contain porosities, so the difference in performance may not be related to the presence of porosity. These porosities did not interfere with measurement of demineralization.

Both Cention N and the experimental material were used with an adhesive. An adhesive was used was due to clinical trials suggesting Cention N and a previous version of Activa BioACTIVE-Restorative show superior performance when an adhesive is used, specifically improved marginal integrity and retention.^{40,41} *In vitro* testing has shown that placement of an adhesive prior to application of fluoride-releasing materials can prevent the demineralization inhibition effect of the fluoride-releasing material.^{19,25} Perhaps the adhesive used in this study could have prevented the restorative materials from inhibiting demineralization in the surrounding tooth structure. In the previous artificial caries

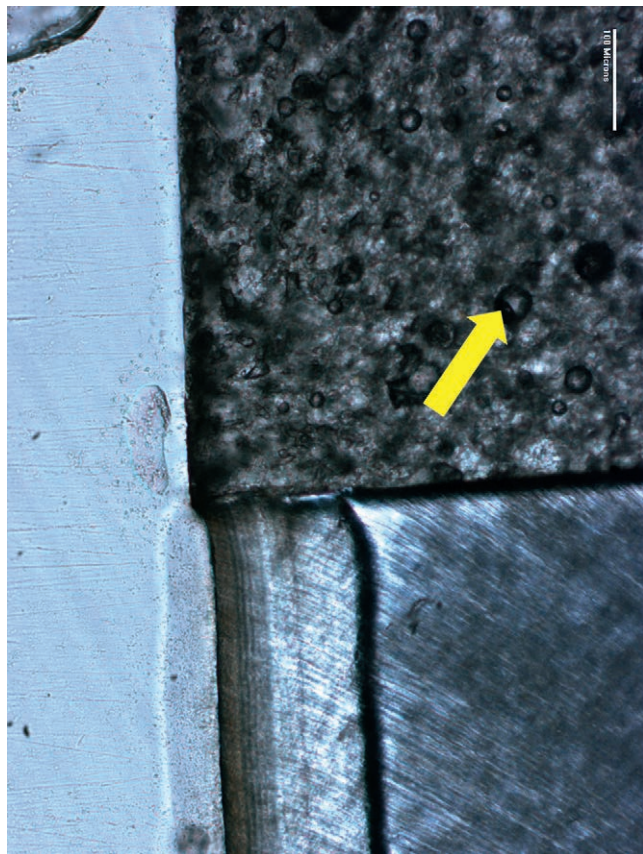


Figure 4. Representative specimen showing an area of inhibition in root dentin (bottom) adjacent to Centon N (a calcium releasing restoration) (top). Arrow indicates a pore within the material.

testing performed with Centon N, the authors did not mention if an adhesive was used.³⁷

There is no standardized method for performing artificial caries testing, and the methodology used in different studies has varied. The pH cycling regimen used in the present study was based on a previous published regimen²⁷ originally credited to Featherstone.³⁸ In the present study, lactic acid was chosen for the demineralization solution rather than acetate. In some studies, specimens have been placed in acidic solutions only (pH=4-5) for between 3 days and 10 weeks.^{19,21,22,24,28} Other studies have performed pH cycling between a pH of 4.5 or 5 and 7.^{20,27} Other studies produced demineralization through exposure to a bacterial caries model. In these studies, the resin composites were placed in a broth containing *Streptococcus mutans* and lactobacillus.^{22-25,27} The results of some studies suggest that bacterial models produce similar results as pH cycling models; whereas, others show different outcomes. For example, a study determined that glass ionomer and RMGI materials provided protec-

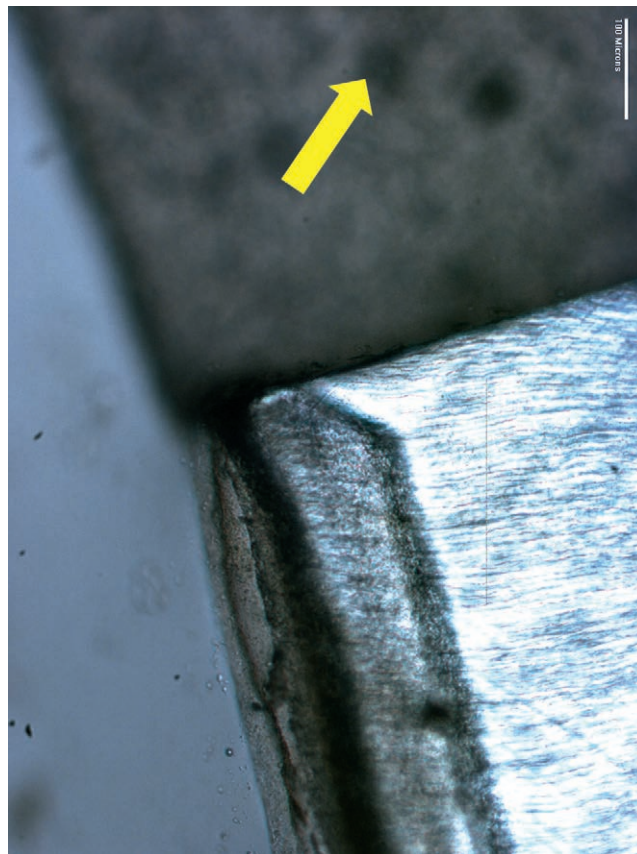


Figure 5. Representative specimen showing an area of inhibition in root dentin (bottom) adjacent to a Fuji II LC [resin-modified glass ionomer (RMGI)] restoration (top). Arrow indicates a pore within the material.

tion from demineralization in a pH cycling model; however, glass ionomer or RMGI materials performed similarly to traditional resin composites, if the demineralization was caused by a bacterial caries model.²² In contrast, another study showed that glass ionomer and RMGI materials showed more protection from demineralization than resin composite in both a bacterial caries model and a pH cycling caries model.²⁷

The methodology used to measure demineralization varied between studies. Most studies measured demineralization through polarized light microscopy^{19,20,21,24}; however, it can also be measured with confocal microscopy²² or microradiography.²³⁻²⁵ The measurement of demineralization has usually included either outer wall lesions,^{21,26,27} areas of wall lesions/inhibition, or both.^{19,20,22,24,25} Outer wall lesions describe the amount of demineralization present from the external surface of the tooth to the internal limit of demineralization. Often the outer wall lesion is measured at a point 100-200 microns away from the restoration margin. Outer

wall lesions are meant to describe the amount of protection provided by a restorative material at a location distant to its interface with the tooth. On the other hand, areas of wall lesion or inhibition are the areas immediately next to the restoration that are measured as described in the methodology of this study. Other methods have been used to estimate the area of a wall lesion or inhibition, such as measuring the linear distance (pulpal-external) of demineralization near the restoration margin²² or estimating the thickness (occlusal-gingival) of the wall lesion or area of inhibition.^{24,28} Measurement of the exact area of inhibition or wall lesion, as performed in the present study, provides information of both the thickness and the depth of these areas. In the present study, the wall lesions and areas of inhibition were measured relative to the front line of the zone of demineralization rather than the external surface of the tooth. This decision was made because teeth experienced different depths of their zone of demineralization relative to their external surface, which could be related to the properties of the tooth rather than the restorative material.

Several technique variables were determined to be critical toward achieving observable lesions within the specimens. The most critical factor was accurately monitoring the pH of the demineralization solution. Due to neutralization from excess water surrounding specimens or possibly from the specimens themselves, the pH of the solution required frequent adjustment by the addition of lactic acid. Daily calibration of the solution was necessary to maintain a constant pH. Possibly the use of acetate to produce an acidic pH would have helped to better buffer the solution. Another critical factor was the volume of solution used per specimen. Through pilot testing, a solution of 500 mL could produce lesions when 40 specimens were immersed within it. A lesser volume of solution with a similar number of specimens would not create consistent demineralization lesions. Alternatively, specimens from each group could have been stored in separate containers of 125 mL, assuming the specimens would be completely immersed in liquid, while the liquid is being stirred.

Future studies should examine the caries inhibition produced by calcium-releasing materials placed with and without a dental adhesive. Although it would not be prudent to use these materials clinically without adhesive,^{40,41} this test methodology could determine whether calcium-releasing materials offer additional benefit from fluoride-releasing materials without the confounding factor

of an intermediary adhesive. Another future study could explore the development of an adhesive that would not prevent any protective effects of ion-releasing materials. The artificial caries model could also be modified such that the remineralization solution contained fluoride in order to simulate the effects of topical fluoride application (ie, fluoridated toothpaste). Previous protocols have incorporated fluoride into the remineralization (0.05 ppm) and demineralization (0.03 ppm) solutions²⁷ or applied topical fluoride as part of the daily cycle.³⁸ Finally, future studies could monitor the progression of demineralization by removing, sectioning, and viewing teeth at various time points.

CONCLUSIONS

In this study, two calcium-releasing materials used with an adhesive provided more protection from root dentin demineralization than a resin composite used with an adhesive; however, these materials provided less protection than a RMGI material used without an adhesive. These findings suggest that calcium-releasing materials, sometimes referred to as bioactive materials, may provide additional utility beyond that of bonded resin composites for prevention of demineralization around restorations. A RMGI material used without an adhesive still provides superior protection from tooth demineralization.

Conflicts of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this manuscript.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of University of Alabama at Birmingham Institutional Review Board (IRB). The approval code issued for this study is 300001291.

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Nanofiller Particles and Bonding Durability, Water Sorption, and Solubility of Universal Adhesives

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Clinical Relevance statement

The commercial universal adhesive containing silanized nanofiller particles decreased dentin bond durability and increased water sorption, which could be reversed by using unfilled adhesive.

SUMMARY

The aim of this study was to evaluate the influence of nanofiller particles in simplified universal adhesive on the long-term micro-

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tensile bond strength and silver nitrate uptake, as well as water sorption and solubility. Commercial adhesives Ambar Universal (FGM) in nanofilled-containing version (filled) and same lot without fillers (unfilled) were donated and applied by means of etch-and-rinse strategy. Microtensile bond strength was surveyed after 24-hours or 1-year water storage. Silver nitrate uptake was assayed using scanning electron microscopy (SEM). Water sorption and solubility experiments were performed based on ISO 4049:2009. Statistical analysis was performed using two-way ANOVA and Tukey test ($p < 0.05$). The bond strength of both the adhesives were statistically similar at 24 hours ($p > 0.05$), but the filled group attained significant bond strength reduction after aging when compared to initial bond strength ($p < 0.001$). Conversely, unfilled adhesive presented stable adhesion after 1-year storage ($p = 0.262$). Silver nitrate uptake was similar for both adhesives, with little silver impregnation at the hybrid and adhesive layers. Water sorption was higher with filled adhesive compared to the unfilled one ($p = 0.01$). Conversely, solubility was higher in unfilled in comparison to filled one ($p = 0.008$). The presence of nano-

fillers in universal adhesive achieves higher water sorption and dentin bond degradation, which did not occur in the unfilled adhesive.

INTRODUCTION

Chewing stresses in the oral environment clinically challenge the bonding interface between resin composite and adhesive. Although dental adhesives have satisfactory initial bonding to dental substrates, these materials may fail after relatively short-term aging periods.^{1,2} Among etch-and-rinse and self-etch adhesives, the etch-and-rinse bonding mechanism relies on micromechanical retention triggered by phosphoric acid etching's superficial dissolution of enamel or dentin.³ In the self-etch approach, the chemical bond of functional monomers with calcium and hydroxyapatite plays a further role in dentin/enamel bonding along with micromechanical interlocking. Furthermore, simplified self-etch adhesives evolved to modern multimode universal adhesives, which may be applied in both bonding strategies.^{4,5}

The acid etching procedure requires effective dentin humidity control to result in optimal penetration of monomers into the interfibrillar spaces of the collagen matrix,³ in order to diminish hydrolytic degradation. However, most hydrophilic monomers are mono-methacrylates, thereby presenting linear polymer chains being more prone to leaching and hydrolysis.⁶⁻⁸

Although, not all simplified adhesive systems contain nanofiller particles, by analogy with restorative resin composites, the addition of nanofillers increases the mechanical properties of the adhesive material itself.^{9,10} However, when the immediate bond strength to dentin was evaluated, controversial results were observed.^{11,12} Actually, the addition of these particles increases the material viscosity while decreasing the polymerization stresses and shrinkage when compared to unfilled adhesives.¹³

One concern is related to the coating of these nanofillers with relatively hydrophilic silane to bond the polymer chains to the fillers. Such silane coating has lower resistance to hydrolysis and may cause the detachment of fillers after aging *in vitro*, which was even found *in vivo*.^{1,2,6}

However, a recent systematic review of clinical trials showed that no significant improvement in terms of clinical performance of the adhesive restoration in noncarious cervical lesions when micro-/nanofilled and unfilled adhesives were compared.¹⁴ Indeed, there are contradictory outcomes

and lack of resin–dentin long-term water storage evaluation on this issue. Particularly, an adequate study could highlight the actual influence of fillers on water uptake and dentin bond durability, when the same composition of simplified universal adhesive with or without nanofiller particles is evaluated.

Therefore, the aim of this study was to survey the influence of nanofillers on the same universal adhesive, ie, nanofilled-containing (filled) and unfilled (unfilled), after 24 hours or 1 year of water storage. The null hypothesis was that the presence/absence of nanofillers will not interfere with dentin bond strength and silver nitrate uptake, as well as with water sorption and solubility of the adhesive material.

METHODS AND MATERIALS

Sample Preparation

The Ambar Universal adhesive (FGM, Joinville, Brazil) was provided by the manufacturer in a nanofiller-containing commercial version (filled, batch number 040218) and the same lot without nanofiller particles (unfilled). Both adhesives were applied and light cured in the same manner, for standardization purposes. Three laboratory experiments were performed: microtensile bond strength after 24-hours or 1-year water storage, silver nitrate uptake, water sorption, and solubility.

Microtensile Bond Strength Testing (μ TBS)

Twenty extracted third molars were divided into two groups ($n=10$). The teeth were stored in 0.5% thymol/water solution at 4°C for no longer than 4 months. They were cut to expose mid-coronal flat dentin surfaces and to remove the roots by means of a diamond saw in a metallographic cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA). Dentin surfaces were polished with 320-grit SiC abrasive papers under running water for 30 seconds to create standardized smear layers. All teeth were bonded following etch-and-rinse strategy. They were etched for 15 seconds with 37% ortho-phosphoric acid (Condac 37, FGM), washed vigorously for 30 seconds with distilled water, and dentin was kept visually moist by using blot-wet cotton pellets. Teeth were randomly assigned to one of the two adhesives (filled or unfilled), and the adhesive was actively applied in two coats—rubbing each coat for 20 seconds with a gentle air blast between the coats. Following, the adhesive was light cured for 40 seconds with a DB-685 LED unit (1200 mW/cm², Dabi Atlante, Rio de Janeiro, Brazil). After adhesive curing, five 1-mm-thick increments of resin composite (Opallis, FGM)

were applied horizontally and individually light cured for 40 seconds each. Resin-bonded teeth were sectioned in resin–dentin-bonded sticks (0.9 ± 0.2 mm² cross-sectional area) suitable for microtensile bond strength testing. Sticks were stored immersed in distilled water for 24 hours or 1 year (with monthly water exchange) prior to testing. Afterwards, resin–dentin-bonded sticks were glued to Geraldelli jigs with cyanoacrylate gel (Super Bonder gel; Loctite Henkel, Rocky Hill, USA) and tested in a universal testing machine (DL 2000, EMIC, São José dos Pinhais, PR Brazil) with a 500-N load cell at 0.5 mm/minute crosshead speed. Subsequent to the μ TBS testing, the failure mode of each fractured stick was determined using a stereomicroscope with 100 $\times$ magnification (Olympus Sz 40–50, Tokyo, Japan). The fractures were classified as adhesive, mixed, cohesive in composite or cohesive in dentin. After passing normality and homoscedasticity tests (data not shown), the μ TBS data were statistically analyzed using two-way ANOVA (presence of fillers and aging period) and Tukey test at $\alpha = 0.05$.

Silver Nitrate Uptake Evaluation

Two resin–dentin-bonded sticks from the central region of each bonded tooth were selected from each subgroup ($n=20$) and processed for silver nitrate uptake assessment, as previously described.¹⁵ In brief, resin–dentin-bonded sticks were immersed in 50 wt% ammoniacal silver nitrate [$\text{Ag}(\text{NH}_3)_2\text{NO}_3(\text{aq})$] solution in total darkness for 24 hours. Subsequently, the specimens were rinsed with distilled water to remove excess silver nitrate and then immersed in a photo-developing solution for 8 hours under fluorescent light (60 cm from the specimens) to reduce silver ions into metallic silver grains along the resin–dentin interface. The silver-impregnated sticks were embedded in epoxy resin and wet polished using #600, #1200, #2000 SiC papers and diamond pastes 3, 1, and 0.25 μm (Buehler) in polishing cloths. The specimens were ultrasonically cleaned for 20 minutes after each abrasive/polishing step. Finally, they were air dried, dehydrated for 48 hours, coated with carbon and observed using scanning electron microscopy (SEM) (Inspect 50, FEI, Amsterdam, Netherlands) in backscattered electron mode to evaluate silver impregnation.

Water Sorption and Solubility

The water sorption and solubility experiments were performed based on ISO 4049:2009 protocol.¹⁶ Ten disk-shaped specimens (6.0 ± 0.1 -mm diameter $\times$ 1.0 ± 0.1 -mm thickness) were prepared for each material ($n=10$). The specimens were created in

standardized silicone molds and were individually light cured using a DB685 LED unit (Dabi Atlante) with 1200 mW/cm² irradiance for 40 seconds. After 24-hour storage in darkness, the specimens were placed in a desiccator containing silica gel and stored at 37°C under vacuum.

The specimens were weighed repetitively using an analytical balance with 0.00001 g precision (Mettler-Toledo AG285, São Paulo, SP, Brazil) each 24 hours, until a constant mass was obtained at which the variation was less than 0.2 mg in a 24-hour period (m_1)¹⁵. The thickness and diameter of the specimens were measured at three different points to the nearest 0.01 mm using a digital caliper. These measurements were used to calculate the volume (V) of each specimen (in mm³). The specimens were then placed in individual microtubes containing 1.5 mL of distilled water at 37°C for 7 days. The microtubes were then removed from the incubator and left at room temperature for 30 minutes. The specimens were rapidly cleaned with soft absorbent paper and weighed in the analytical balance (m_2). Thereafter, the specimens were dried in the silica gel-containing desiccator and weighed daily until a constant mass (m_3) was obtained (as described above). Changes in mass were observed to obtain water sorption and solubility. Water sorption (W_{sp}) and solubility (W_{sl}) were calculated ($\mu\text{g}/\text{mm}^3$) by the following formulas:

$$W_{\text{sp}}: \frac{M_2 - M_3}{Vol}$$

$$W_{\text{sl}}: \frac{M_1 - M_3}{Vol}$$

Where M_1 refers to the initial dry constant mass (mg) before immersion in water; M_2 is attributed to the mass (mg) after immersion in water at various time periods; M_3 is the mass (mg) after the final drying of the specimens, and Vol refers to the sample volume (mm³)¹⁵.

After passing normality and homoscedasticity tests (data not shown), the W_{sp} and W_{sl} data were statistically analyzed using one-way ANOVA (presence of fillers) and Tukey test with a 5% significance level.

RESULTS

Microtensile bond strength (μ TBS)

The outcomes of microtensile bond strength are shown in Table 1. Both the adhesives depicted

Table 1: Means (Standard Deviations) of Microtensile Bond Strength (MPa)^a

	24 Hours	1 Year
Filled	48.9 (3.4) Aa	17.2 (2.6) Bb
Unfilled	46.6 (8.6) Aa	43.0 (5.8) Aa

^a Different capital letters in each column and lowercase letters in each row indicate statistically significant difference ($p < 0.05$).

similar bond strength at the 24-hour period (filled= 48.9 ± 3.4 MPa and unfilled= 46.6 ± 8.6 MPa). After 1 year of water storage, the unfilled adhesive (43.0 ± 5.3 MPa) was statistically similar to the 24-hour period, but the filled group (17.2 ± 6.2 MPa) demonstrated significant bond strength reduction ($p < 0.001$).

The fracture patterns were predominantly similar and most frequently occurred in adhesive mode for all groups. However, the unfilled adhesive showed some cohesive failures in resin composite at both periods of storage. Fracture pattern outcomes are presented in Table 2.

Silver Nitrate Uptake Evaluation

The silver nitrate uptake micrographs of specimens showed similar amounts of silver deposits at interfaces of both the adhesives (Figure 1). The filled group depicted small gaps between the adhesive layer and resin composite, as well as silver impregnation in the middle of adhesive layer (Figures 1A and 1B). Although, the interfaces created in unfilled group (Figures 1C and 1D) showed more silver deposits located at the hybrid layer.

Water Sorption and Solubility

Table 3 shows the mean results of water sorption and solubility. There was higher W_{sp} for the filled adhesives (5.88 ± 0.97 $\mu\text{g}/\text{mm}^3$) compared to unfilled adhesive (2.03 ± 1.09 $\mu\text{g}/\text{mm}^3$), with a statistically significant difference between them ($p = 0.01$). Contrariwise, W_{sl} was higher in the unfilled adhesive (8.93 ± 1.83 $\mu\text{g}/\text{mm}^3$) compared to the filled adhesive

Table 3: Means (Standard Deviations) of Water Sorption and Solubility ($\mu\text{g}/\text{mm}^3$)

	Water Sorption	Solubility
Filled	5.88 (0.97) B	2.94 (1.00) a
Unfilled	2.03 (1.09) A	8.92 (1.82) b

^a Different capital letters for water sorption results and different lowercase letters for solubility outcomes indicate statistically significant difference ($p < 0.05$).

(2.94 ± 1.01 $\mu\text{g}/\text{mm}^3$), with a statistically significant difference between them ($p = 0.008$).

DISCUSSION

Multiple methods are used to assess the longevity of bonding interfaces. This study performed the storage of resin–dentin-bonded sticks in distilled water during 1 year for microtensile bond strength assessment of bonding durability. The long-term bond strength decreased significantly only for the filler-containing adhesive. Moreover, water sorption and solubility were significantly different between the adhesives. Therefore, the null hypothesis should be rejected.

In the present investigation, the same batch of commercial universal adhesive was investigated, before or after the inclusion of silanized nanofiller particles. In order to standardize the etch-and-rinse bonding technique and to provide optimal initial bond strength values, the blot-drying cotton pellet technique was employed, according to previous literature.^{17,18} It is noteworthy that the immediate bond strength of both the groups did not have any significant difference. These results are in agreement with previous studies that evaluated old versions of simplified etch-and-rinse adhesives.^{11,12}

In terms of simplified adhesives, nanometer-sized silica smaller than 20 nm are usually added^{19,20} and the amount of nanofiller available in commercial brands usually ranges between 5% and 10% wt%.¹⁰ The addition of nanofillers has two intended functions—to reinforce the adhesive layer and to improve the hybrid layer with the infiltration of nanofillers into dentin tubules and into the collagen network.²¹ Previous studies have shown that simplified adhesives with nanofillers may yield better mechanical properties compared with unfilled adhesive systems.¹² However, the improvement is material dependent.¹²

In terms of the hybrid layer, according to the Bertassoni and others,²² the space between collagen fibrils is within the size of a few nanometers. This space is frequently filled with water, thereby challenging the infiltration of filler particles from

Table 2: Spreading of Outcomes of Fracture Analysis After Microtensile Bond Strength Test^a

Type of Fracture	Filled		Unfilled	
	24 Hours (%)	1 Year (%)	24 Hours (%)	1 Year (%)
Cohesive in Composite	—	—	20	25
Mixed	6	8	4	12
Adhesive	94	92	76	63

^a No cohesive failure in dentin was observed in any group.

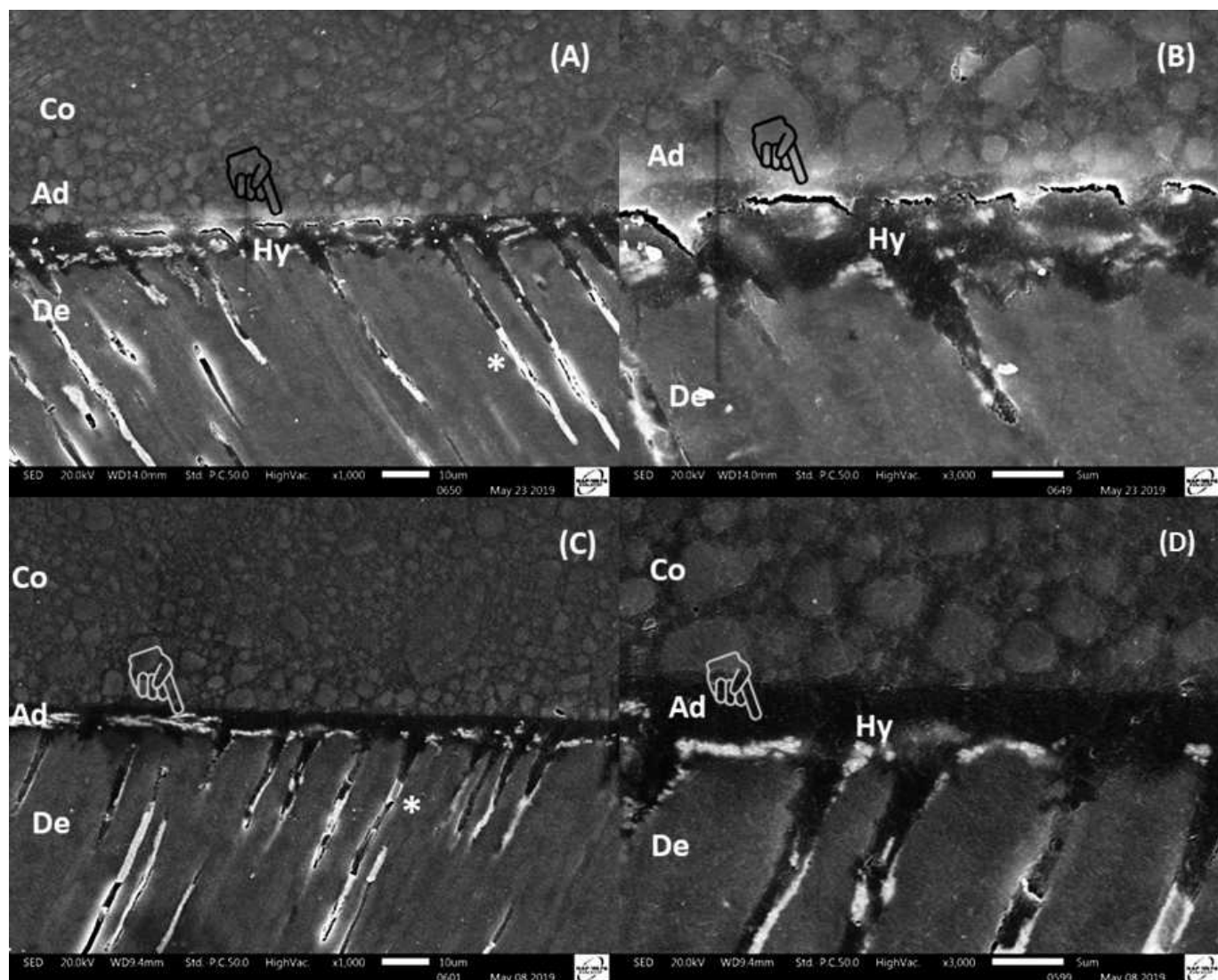


Figure 1. Representative scanning electron microscopy (SEM) images of the silver nitrate uptake at 1000 $\times$ and 3000 $\times$ magnification. Ambar Universal adhesive with presence of fillers at 1000 $\times$ (A) magnification and (B) at 3000 $\times$. (C) Unfilled adhesive suggests presence of water in the hybrid layer in 1000 $\times$ magnification and 3000 $\times$ (D). Co = resin composite; Ad = adhesive; De = dentin; Hy = hybrid layer. White arrow identifies the presence of silver at the bonding interface. Black arrow identifies the presence of gap in the resin/bond-bonding interface. * Represents presence of water in the dentinal tubules.

adhesive into the hybrid layer properly. Unfortunately, several studies showed that no nanofiller had been found inside the hybrid layer or in the demineralized dentin,^{1,21,23} mainly because the nanofiller has a tendency for aggregation, forming “filler clusters” as observed in the Figures 1A and 1B. These clusters are too large to infiltrate the collagen interfibrillar spaces.^{21,24}

It is highlighted herein that a significant drop of bond strength occurred with nanofiller-containing adhesive (Table 1). On the other hand, there was bonding stability for the nanofiller-free adhesive. Indeed, this suggests that the presence of nanofiller

particles is one of the triggering factors for degradation of resin–dentin interfaces, which may be an alternative to disregard the use of such nanoparticles in order to improve the durability of resin composite restorations. Nevertheless, when nanofiller particles are added to adhesive systems, the addition of a silane coupling agent is needed, which functions to promote the binding of filler particles to polymeric chains but also possesses a relatively hydrophilic nature.¹⁰

Therefore, the presence of a silane coating lowers the resistance to hydrolytic degradation due to the breakdown of silanol and the hydrolysis of ester

bonds from methacrylate in the presence of water.^{25,26,27} Water infiltration initiates the hydrolysis of silane at the filler–polymer interfaces, thereby acting as the threshold for filler debonding and weakening of resin–dentin bonds, particularly causing the leaching of fillers.^{1,2,6} Indeed, this could be confirmed by the fact that nanofiller-containing adhesive yielded higher water sorption when compared to unfilled adhesive. Yet, the presence of fillers may diminish formation of crosslinked polymeric matrix by steric impediment.^{28,29}

Water seepage, polymer hydrolysis, and consequent degradation cannot be avoided, although it must be considered that in the present investigation the tiny resin–dentin-bonded sticks samples were directly stored in distilled water,^{30,31} which may have increased detachment of fillers and leaching of monomers with accelerated aging of dentin–adhesive interface.^{32,33}

Interestingly, although an increase in water sorption occurred for filled adhesive, the solubility decreased for filled adhesive when compared to unfilled. A suitable explanation for such an outcome is related to the presence/absence of filler particles. Solubility occurs only from resin matrix and is absent in inorganic fillers, which are insoluble. Therefore, unfilled adhesive attains higher solubility, because fillers are added usually in 5–10 wt% to filled adhesive, and these fillers do not participate in solubility of the bulk material. In other words, only 90–95% of the material is soluble in filled adhesive, whereas the unfilled adhesive possesses the entire (100%) material prone to solubilization. Thus, unfilled adhesive was indeed expected to achieve higher solubility in comparison to filled adhesive. Nevertheless, novel silane coatings have been developed to minimize the effects of hydrolytic degradation of the coupling agent by decreasing the binding strength of water to SiOH and SiO functionalities.^{34,35}

In addition to the degradation of silane, water causes degradation of methacrylate in polymer chains. Monomers such as HEMA (hydroxyl-ethyl methacrylate) are often employed in simplified universal adhesives. However, HEMA is highly susceptible to water sorption and leaching due to its low molecular weight, mono-methacrylate structure, and hydrophilic feature.^{32,36} Ambar adhesive (a predecessor of Ambar Universal) has shown optimal mechanical properties as compared to further commercial simplified adhesives.^{37–39}

The silver nitrate uptake images revealed signs of bonding failure (small gaps) in the filled group and

noteworthy presence of silver impregnation in the bonding interface of the unfilled group (Figure 1). These features are commonly observed in resin–dentin interfaces created with the most universal adhesives.³⁹ In addition, the greater presence of silver in the adhesive layer of the filled group is noticeable, which may be explained by the clustering of fillers (Figure 1B).

Although the vast majority of commercial adhesives contain fillers in their composition, the present investigation suggests their presence may impair optimal dentin bond durability by increasing the water sorption. Therefore, if manufacturers still demand addition of fillers to universal adhesives, new investigations should focus on the development of hydrolysis resistant hydrophobic silane molecules to maintain the benefits on adhesive mechanical properties as well as using specific techniques to produce nonaggregated nanoparticles.^{41,42} Particularly, we suggest the addition of fillers with antimicrobial and remineralizing characteristics.^{42,43}

CONCLUSIONS

Within the limitations of this investigation and considering that a single universal adhesive was surveyed, one may draw the conclusions that the presence of nanofiller particles in a commercial universal adhesive, applied in etch-and-rinse bonding strategy, may lead to loss of bond strength to dentin and higher water sorption compared to the same unfilled material.

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Regulatory Statement

IRB approval: “This study was conducted in accordance with all the provisions of the human subjects’ oversight committee guidelines and policies. The approval code issued for this study is 011133/2018.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Departments

Faculty Positions



College of Dentistry

Postgraduate Program Director, Advanced Prosthodontics

Advanced Prosthodontics Program
Department of Restorative Dentistry
University of Illinois at Chicago
College of Dentistry

The University of Illinois Chicago College of Dentistry (UIC COD) invites applications for a full time faculty to serve as the Program Director for the Advanced Education Program in Prosthodontics. This position is **Open Rank either in Tenure or Non-Tenure Track** in the Department of Restorative Dentistry. This is an exceptional opportunity to lead a team of outstanding residents, faculty and staff in a state of the art Prosthodontics facility.

We are seeking an experienced academic leader in prosthodontic education, who will build upon our established educational foundation and continue the mission of the Department and the College. Additionally, the individual should possess excellent organizational and interpersonal skills, experience in mentoring and leadership, and a commitment to diversity and inclusion.

The selected individual will have primary responsibilities in the recruitment, training, and supervision of postgraduate students specializing in prosthodontics. The Program Director will also oversee strategic planning, curriculum organization, financial management and ensure that the program complies with the Commission on Dental Accreditation standards.

Candidates must have earned a DDS/DMD (or equivalent) degree, be eligible for licensure in Illinois, must have completed a formal training program in prosthodontics at a CODA accredited institution and must be a diplomate of the American Board of Prosthodontics. Preferred candidates will have experience/formal training in dental implant surgery;

experience/formal training in digital dentistry, experience teaching in an advanced prosthodontics program, experience as a research mentor at the advanced prosthodontics level and evidence of national leadership in advanced prosthodontics. Academic rank and salary will be commensurate with qualifications and experience.

The position is available immediately. Confidential review of materials and screening of candidates will begin in January of 2022 and continue until the position is filled. For fullest consideration, please apply by February 1, 2022 through the university website at: <https://jobs.uic.edu/job-board/job-details?jobID=158102> (tenured) or <https://jobs.uic.edu/job-board/job-details?jobID=158094> (non-tenure) including cover letter, curriculum vitae, license, and the names and contact information of four professional references. Inquiries regarding this position may be addressed to Dr. Stephen Campbell, Search Committee Chairperson at stephend@uic.edu.

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Mechanical Stability and Proteolytic Activity of Resin–dentin Bonds Using the Cross-linked Dry Bonding Technique

M Citta • G Anovazzi • FG Basso • DLS Scheffel
J Zhou • DH Pashley • CA Souza Costa • J Hebling

Dentin collagen cross-linking inhibits proteolytic activity, increases the stability of hybrid layers, and improves collagen resistance to air drying, thereby, eliminating the influence of dentin wetness status on dentin bonding, even if a water-free adhesive is used.

<http://doi.org/10.2341/20-016-L>

***In Vitro* Biocompatibility of CPP-ACP and Fluoride-containing Desensitizers on Human Gingival Cells**

S López-García • J Guerrero-Gironés • MP Pecci-Lloret
MR Pecci-Lloret • FJ Rodríguez-Lozano • D García-Bernal

Different commercial desensitizers with the same therapeutic indication exhibit different biological effects and cytotoxicity on human gingival fibroblasts, which could be considered criteria for choosing the optimal treatment of dentin hypersensitivity.

<http://doi.org/10.2341/20-245-L>

Challenging the Concept that OptiBond FL and Clearfil SE Bond in NCCLs Are Gold Standard Adhesives: A Systematic Review and Meta-analysis

FDS Dreweck • A Burey • M de Oliveira Dreweck • E Fernandez • AD Loguercio • A Reis

The concept of gold standard dental materials should be re-evaluated. The evidence in the literature does not support the stating that OptiBond FL and Clearfil SE Bond are better than other materials in NCCLs.

<http://doi.org/10.2341/20-059-LIT>

Effects of Remineralizing Agents Based on Calcium Phosphate, Sodium Phosphate, or Sodium Fluoride on Eroded Cervical Dentin

WG Escalante-Otárola • GM Castro-Núñez • TP Leandrim
CM Alencar • FF de Albuquerque Jassé • MC Kuga

Calcium phosphate-based remineralizing agents can prevent the harmful effects of erosion challenges on the dentin collagen matrix. In addition, they may be a promising and viable alternative for treatment of dentin hypersensitivity—and prevent the harmful impacts of erosion challenges on the collagen matrix.

<http://doi.org/10.2341/20-209-L>

Three-dimensional Change of Elastomeric Impression Materials During the First 24 Hours: A Pilot Study

H Roberts

Clinicians should be aware that volumetric polymerization shrinkage of elastomeric impression material continues for up to 24 hours after mixing. More accurate results may be obtained if impressions are poured as early as possible following the manufacturer's recommendations.

<http://doi.org/10.2341/20-265-L>

Mechanical Stability and Proteolytic Activity of Resin–dentin Bonds Using the Cross-linked Dry Bonding Technique

M Citta • G Anovazzi • FG Basso • DLS Scheffel • J Zhou
DH Pashley • CA Souza Costa • J Hebling

Clinical Relevance

Dentin collagen cross-linking inhibits proteolytic activity, increases the stability of hybrid layers, and improves collagen resistance to air drying, thereby, eliminating the influence of dentin wetness status on dentin bonding, even if a water-free adhesive is used.

SUMMARY

Objective: To evaluate the mechanical stability and the proteolytic activity of bonds created by a two-step, etch-and-rinse adhesive applied to cross-linked and air-dried etched dentin.

Methods: Flat dentin surfaces were produced in

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64 extracted sound human molars. The dentin was etched with 35% phosphoric acid for 15 seconds, and then the teeth were divided into groups according to the cross-linking solution applied on the etched dentin. Group 1: 5% grape seed extract (GSE), Group 2: 5% glutaraldehyde, Group 3: Gluma Desensitizer, or Group 4: deionized water

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(control). Solutions were applied for 60 seconds, followed by rinse and blot drying. Then, the teeth were separated into two subgroups where the etched dentin was kept moist or air-dried. The adhesive was applied followed by a composite resin buildup. After 24 hours, the teeth were cut into beams (0.81 mm²) that were tested for microtensile strength immediately or after 12 months of aging in a 37°C saliva-like buffer. Additional teeth (n=32) were bonded as described and cut into 0.5-mm-thick slabs. The slabs were prepared for nanoleakage (scanning electron microscopy) and *in situ* zymography (EnzChek Protease Assay Kit). Bond strength data were submitted to ANOVA and Tukey tests ($\alpha=0.05$).

Results: Significant reduction in immediate bond strength (ca 65%) and increase in proteolytic activity was seen when the etched dentin was air dried without previous cross-linking biomodification. Conversely, bond strengths did not differ from those produced on wet dentin when collagen was cross-linked before air drying, irrespective of the solution applied. For both moist and air-dried etched dentin, collagen cross-linking resulted in mechanically stable bonds and reduced proteolytic activity after 12 months of storage.

Conclusion: Bonds produced by the application of a two-step, etch-and-rinse adhesive to cross-linked, air-dried, etched dentin were mechanically stable and revealed reduced proteolytic activity after 1 year of aging.

INTRODUCTION

The hybrid layer is a microscopic structure that serves two fundamental roles in dentin adhesion—retention of the restorative material and sealing of the intrinsically wet collagen of the dentin matrix. The ideal hybrid layer should contain an impermeable three-dimensional structure composed of the mixture of polymer and collagen, capable of guaranteeing a continuous and stable bond between the dentin and the adhesive material.¹ However, this biocompound contains imperfections created during its formation, such as areas with poor monomer infiltration, hydrophilic and hydrophobic moieties,² retention of water and solvent, and the presence of water-filled denuded collagen fibrils.³ As a result, despite its utmost importance, the hybrid layer has been considered the weak link in resin–dentin bonds.¹ Complete infiltration of the acid-etched dentin by adhesive monomers^{3–8} is hindered by

residual water between the collagen molecules.⁹ Two main factors limit monomer infiltration into these nanospaces: the presence of free water molecules and water molecules bound to demineralized collagen,¹⁰ and the dense packing of the collagen molecules.^{9,11–12}

Deterioration of hybrid layers occurs irrespective of the adhesive system used; that is, it occurs in etch-and-rinse and self-etching adhesives,^{12–15} and involves hydrolysis of the resin polymer and the breakdown of the exposed collagen—the two main components of this biocompound.^{11,12,15} The degradation of the exposed collagen within the hybrid layer by intrinsic proteolytic enzymes bound to collagen is claimed to be the cause of the failure of interfaces produced on dentin.^{16,17}

After acid etching dentin, water is important for maintaining the interfibrillar spaces that serve as a pathway for infiltration of the monomers of the adhesive.¹⁸ At the same time, water limits the infiltration of hydrophobic monomers, compromises monomeric conversion,^{19,20} and allows hydrolysis of the collagen mediated by proteolytic enzymes such as the matrix metalloproteinases (MMPs) and cysteine cathepsins.^{11,12} Nevertheless, attempts to remove the residual water content of etched dentin simply by air evaporation causes collapse of the collagen fibrils and, consequently, loss of the interfibrillar spaces needed for the infiltration of the adhesive monomers.^{18,21–23} The stiffness of mineralized dentin is about 20 MPa, which makes it almost incompressible, whereas the stiffness of demineralized dentin is about 0.1 MPa,²⁴ which is similar to that of cooked spaghetti.

Biomodification of demineralized dentin with cross-linkers has been used to increase the mechanical properties of demineralized dentin collagen to make it more resistant to proteolytic degradation.^{25–28} Commonly used cross-linkers are carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)], glutaraldehyde, and polyphenols such as proanthocyanidins (PAs). These agents increase the strength and stiffness of both collagen molecules and collagen fibrils.^{24,26,29,30} Also, these agents are powerful inhibitors of matrix metalloproteinases (MMPs) and cysteine cathepsins (CATs), even when applied on the demineralized dentin for only 60 seconds.^{31–33} The basic mechanism of cross-linkers is to increase the number of cross-links at the intramolecular, intermolecular (or intrafibrillar), and interfibrillar levels.^{34,35}

It has been reported that once biomodified by a proanthocyanidin-rich extract [grape seed extract (GSE)], the reinforced collagen can resist air drying without shrinking and with no detrimental consequences to the immediate bond strength.²³ Conversely, without prior cross-linking of collagen, the immediate bond

strength was significantly reduced after prolonged air-blast drying of the dentin. This new adhesive approach was called cross-linked dry bonding.²³ However, the resistance of cross-linked collagen against air drying was evaluated only 24 hours after bond formation. Its long-term validation is still necessary, since the main challenge of bonding to dentin is the creation of hybrid layers resistant to the mechanisms of hydrolytic and enzymatic degradation over time.

Therefore, the aim of the study was to evaluate the mechanical stability and the proteolytic activity of resin–dentin bonds created by a water-free, two-step, etch-and-rinse adhesive applied to cross-linked, air-dried etched dentin, in comparison to bonds produced using the conventional wet-bonding technique. The null hypotheses tested were that the interfaces produced on biomodified and dry dentin (cross-linked dry bonding) do not differ from interfaces produced using the wet-bonding technique relative to mechanical stability, nanoleakage, and proteolytic activity.

METHODS AND MATERIALS

Specimen Preparation

Sixty-four extracted human third molars were used to evaluate the mechanical stability of interfaces produced on biomodified and dry dentin, using as experimental protocol approved by the Ethics Committee on Research with Human Beings. With a diamond-coated disc coupled to a metallographic cutter (ISOMET 1000, Buehler, Lake Bluff, IL, USA), the enamel and occlusal-third of the tooth crown was removed, exposing a flat dentin surface. The dentin surfaces were manually abraded with 320-grit abrasive paper for 30 seconds, to create a standardized smear layer comparable with those clinically produced with carbide burs.^{36,37} The teeth were then randomly distributed into four experimental treatment groups (n=16): 5% GSE, 5% glutaraldehyde (GD) in water, Gluma Desensitizer, and water (control).

Dentin Treatment and Bonding Procedures

The dentin surfaces were etched with 35% phosphoric acid (Ultradent Products INC, South Jordan, Utah, USA) for 15 seconds, followed by water rinsing for 10 seconds and drying with absorbent paper. Then, the dentin was treated with one of the cross-linkers (Table 1) or with deionized water (control group). Twenty microliters (20 μ L) of 5 wt% GSE, 5% GD, or deionized water (control) were applied on the dentin surface for 60 seconds and then rinsed off with 5 mL of deionized water. Gluma Desensitizer (Heraeus Kulzer GmbH, Hanau, Germany) was applied following the manufacturer's recommendations. Thus, 20 μ L of the product was applied on the etched wet dentin and kept in place for 60 seconds. After this, a jet of air was applied for 5 seconds at a distance of 10 cm from the surface to blow the excess off, and then the surface was washed with 5 mL of deionized water. Then, the teeth of each group were subdivided according to the condition of dentin wetness. In half of the teeth (n=8), the dentin was kept moist by a wet piece of absorbent paper. In the other half (n=8), the dentin surface was dried by a constant jet of air for 60 seconds, with the syringe positioned at a distance of 10 cm from the surface.²³

The adhesive system Optibond S (Kerr Corporation; Orange, CA, USA) was applied on the entire dentin surface and scrubbed for 15 seconds. Subsequently, a jet of air was applied for 3 seconds at a distance of 10 cm, and the adhesive was light activated for 20 seconds (*ca* 1000 mW/cm²; LED light Radii Plus, SDI Limited, Bayswater, Victoria, Australia). A block of resin composite (Filtek Z350, 3M Oral Care, St. Paul, MN, USA) was built up in three increments of 1 mm each, which were individually light activated for 20 seconds at a radiant emittance of 1000 mW/cm². The teeth were then stored immersed in deionized water at 37°C for 24 hours.

Microtensile Bond Strength

After 24 hours, the restored teeth were sectioned with a metallurgical saw (ISOMET 1000, Buehler Ltd, Lake

Table 1: Information About the Composition of Agents Used to Biomodify the Dentin Collagen

Agent	Main Components	Manufacturer	Mixture/pH ^a
5% GD	Glutaraldehyde	Sigma-Aldrich Corp, St Louis, MO, USA	0.5 mL of 50% GD; 4.5 mL of deionized water; pH 6.4
5% GSE	Proanthocyanidin-rich extract from the seed of <i>Vitis vinifera</i>	Shaanxi Sinuot Biotech Co Ltd (Shaanxi, China)	0.25 g of GSE; 5 mL of deionized water; pH = 5.5
Gluma Desensitizer	HEMA (25%-50%) Glutaraldehyde (5%-10%)	Heraeus Kulzer GmbH (Hanau, Germany)	Ready to use; pH = 4.0

Abbreviations: GD, glutaraldehyde; GSE, grape seed extract; HEMA, hydroxyethyl methacrylate.

^aDeionized water pH = 6.2.

Bluff, IL, USA) containing a 0.3-mm thick diamond-coated disc (Diamond Wafering Blade, Buehler Ltd, Lake Bluff, IL, USA), under constant irrigation. A series of 0.9-mm-thick sections were made in both x and y directions to produce rectangular beam-shaped specimens, with a cross-sectional area of approximately 0.81 mm². The adhesive areas of these specimens were individually measured with a digital caliper (Mod 500-144b, Mitutoyo Sul Americana Ltda., SP, Brazil) with a resolution of 0.01 mm. The specimens obtained from each tooth were randomly divided into two groups, according to the period of aging. Thus, one group was mechanically tested immediately, and the other group was tested after 12 months of storage at 37°C in a saliva-like buffer solution (12.9 mM KCl, 1.9 mM KSCN, 2.4 mM Na₂SO₄, 3.3 mM NH₄Cl, 1.5 mM CaCl₂, 7.5 mM NaHCO₃, 0.02 mM ZnCl₂, and 5 mM HEPES buffer, pH 7.4).³⁸

The microtensile bond test was performed in a mechanical test machine (DL-Digital Line, EMIC, Paraná, Brazil) equipped with a 100 N load cell, operating at a speed of 1.0 mm/min. The specimens were individually fixed to a metal device with cyanoacrylate adhesive (Super Bond Gel and Activator 7456, Henkel Loctite Ltda, São Paulo, Brazil), and the device was adapted to the test machine. The tensile movements were initiated by means of a specific computerized program (Tesc-Test Script, EMIC Equipamentos de Ensaio Ltda, São José dos Pinhais, Paraná, Brazil) until the specimen ruptured. The maximum load values recorded (N) were divided by the area of each specimen (mm²) to obtain the tensile bond strength values in megapascals (MPa). Immediately after the test, the halves obtained for each specimen were analyzed under a stereomicroscope (Mod SZX7, Olympus, São Paulo, Brazil) at magnifications ranging from 10× to 30×. Failure modes were classified as adhesive, mixed, cohesive in resin, or cohesive in dentin.³⁷

Nanoleakage and *in Situ* Zymography

Thirty-two teeth were used for silver nanoleakage and for *in situ* zymography, which were divided into the same groups and treated the same way as was previously described. Using the metallurgical saw, cuts were made perpendicular to the adhesive bond to obtain 0.5-mm-thick slices. Slices cut from the same tooth were equally distributed for nanoleakage and *in situ* zymography studies, and aging periods (24 hours and 12 months). Therefore, the slice (specimen) was the experimental unit, and the number of repetitions considering the interaction cross-linker versus dentin wetness versus period of aging was n=4 for each protocol.

Nanoleakage and Scanning Electron Microscopy (SEM)—

The 0.5-mm-thick slices were submitted to the nanoleakage protocol, as previously published.³⁹ To rehydrate the specimens, they were kept in distilled water for 20 minutes and afterward immersed in a 50 wt% ammoniacal silver nitrate tracer solution (pH 9.5) in the absence of light for 24 hours. After this, they were washed with deionized water for 5 minutes and immersed in a developing solution (Revelador Kodak, São José dos Campos, São Paulo, Brazil) under a fluorescent light for 8 hours. The specimens were washed in water for 2 minutes and polished with a sequence of silicon carbide abrasive papers of decreasing roughness (400, 600, 1200, 2000, and 4000 grit) moistened with deionized water. Afterward, the slices were rinsed in running water for 3 minutes, dried with absorbent paper, mounted on metal stubs, and stored in a desiccator containing dry calcium sulfate powder for 24 hours. They were imaged by backscattered scanning electron microscopy (SEM) (Inspect S50; FEI Company, Hillsboro, Oregon, USA), and images of the interface were obtained at 1500× magnification.

In Situ Zymography—

For *in situ* zymography of the intrinsic gelatinase activity of bonded dentin, the specimen slices were manually abraded with abrasive papers, 1200 and 4000 grit until a thickness of 0.3 mm was attained. Each slice was then glued to an acrylic slide using SuperBonder (Henkel Ltda, São Paulo – SP) and stored in deionized water at 4°C for 24 hours. The *in situ* zymography was performed using the EnzCheck gelatinase/collagenase Assay Kit (Molecular Probes, Eugene, OR, USA). The fluorescein-labeled DQ gelatin was diluted in buffer 1:8 (NaCl 150 mM, CaCl₂ 5 mM, Tris-HCl 50 mM, pH 8). The gelatin solution was prepared mixing 10 µL of the gelatin stock solution, 80 µL of dilution buffer, and 10 µL of antifade solution. Afterward, 30 µL of the gelatinous solution containing fluorescein were applied on the specimens, which were covered with a glass plate and stored in an oven at 37°C, in 100% humidity, and in the absence of light. After 24 hours of incubation, the samples were evaluated under a fluorescence microscope DM 5500 (ex: 498 nm and em: 530 nm) (Leica Microsystems, Wetzlar, Germany), and photographic images of the interface were obtained at 10× magnification.

Statistical Analysis

For the bond strength data, specimens of the same tooth within each period of aging were averaged using the tooth as the experimental unit. Sample size was

calculated using the software after obtaining the data and considering the bond strength means and standard deviations of the control group for both independent variables (dentin wetness and aging period). Fixed parameters were alpha 0.05, power 0.8, two-tailed test, and $N_2/N_1=1$. The bond strength data were initially submitted to a three-way analysis of variance (ANOVA), considering the factors (1) etched dentin treatment, (2) dentin wetness, and (3) period of aging. Then, significant interactions were analyzed by two-way ANOVA complemented by Tukey tests. The level of significance established was 5%. Nanoleakage and *in situ* zymography results were qualitatively analyzed.

RESULTS

Bond Strength

The bond strength values of the various groups are presented in Table 2. The three-way ANOVA revealed no effect of the interaction of the three factors ($p=0.170$) on bond strength. Conversely, significant effects were shown for etched dentin treatment*dentin wetness ($p<0.001$), etched dentin treatment*aging period ($p<0.001$), and dentin wetness*aging period ($p<0.001$). Therefore, two-way ANOVA was applied to the interactions, and significant differences between groups detected by the Tukey test are given in Table 2.

Without prior cross-linking of the collagen, the immediate bond strength was significantly reduced by 61.7% when the adhesive system was applied on dentin dried with air in comparison with wet dentin.

Cross-linking of the etched dentin with the GSE, GD, or Gluma Desensitizer did not interfere in the immediate bond strength values when the dentin was maintained wet before application of the adhesive. However, increased bond strength was observed when the adhesive was applied on dentin dried with air, which had previously been biomodified by GSE, GD, or Gluma Desensitizer, in comparison with dry dentin that was not biomodified. These values became statistically similar to the values obtained for the control group, that is, dentin that was not biomodified but kept wet before application of the adhesive. Thus, cross-linked dentin could be dried without negative influence on the immediate bond strength.

After 12 months of aging in saliva-like buffer, a significant reduction in the bond strength was observed for both non-cross-linked control groups. This reduction was 37.1% when the dentin was kept wet, and 37.3% when the dentin was dried with air before application of the adhesive. Differently, the bond strength values of the groups treated with GSE, GD, and Gluma Desensitizer remained stable, that is, comparable with the respective groups at the 24-hour aging period. Irrespective of the aging period and dentin wetness, there was no difference in performance found among GSE, GD, and Gluma Desensitizer.

Failure Mode

The two halves of the specimens subjected to microtensile bond testing were examined under stereomicroscopy. The distribution of the fracture types is shown in

Table 2: Bond Strength (MPa) to Wet or Air-dried Etched Dentin with No Biomodification (Water-treated Dentin) or with Previously Biomodified with 5% Grape Seed Extract (GSE), 5% Glutaraldehyde (GD), or Gluma Desensitizer^a

Etched Dentin Treatment	Dentin Wetness	Aging Period ^b	
		24 Hours	12 Months
Water (control)	Wet	43.4 ± 6.4 a	27.3 ± 2.5 Ba*
	Dry	16.6 ± 3.9 b	6.2 ± 1.1 Ca*
5% GSE	Wet	35.5 ± 8.9 a	34.7 ± 5.2 A
	Dry	31.9 ± 3.8 a	30.9 ± 2.5 A
Gluma Desensitizer	Wet	38.5 ± 5.0 a	33.3 ± 7.2 A
	Dry	34.1 ± 5.5 a	33.8 ± 2.6 A
5% GD	Wet	39.7 ± 11.1 a	32.8 ± 5.3 A
	Dry	34.4 ± 10.8 a	37.0 ± 8.5 A

Abbreviations: GD, glutaraldehyde; GSE, grape seed extract.

^aBond strength was evaluated 24 hours and 12 months after storage of bonded specimens in saliva-like buffer.

^bNumbers are mean ± standard deviation, $n = 8$. Within each aging period, means followed by different letters are statistically different (Tukey, $p<0.05$). Means followed by an asterisk (*) in the 12-month aging period column indicate statistically significant difference compared to the 24-hour period of aging.

Figure 1. Adhesive fractures were predominant in all groups, regardless of the treatment of the etched dentin, dentin wetness, or period of aging. In the water-treated etched dentin (Figure 2a), there was an increase in the adhesive-type failure in interfaces produced on dry dentin (90.6%) compared to the dentin (73.3%) at 24 hours. After 12 months of aging, almost all the interfaces failed adhesively (94% for wet dentin and 97% for dry dentin) (Figure 2a). This pattern was not repeated in the groups in which the etched dentin was cross-linked with 5% GSE (Figure 2b), 5% GD (Figure 2c), or Gluma Desensitizer (Figure 2d). When the interfaces of biomodified wet or dry dentin were examined, there was little influence on the distribution of fractures.

Nanoleakage

Silver uptake was observed in all the bonded adhesive interfaces, even after only 24 hours of storage. In that period of aging (Figure 2), drying the etched dentin with air before applying the adhesive resulted in a

considerable increase in silver uptake, in comparison with the wet control (Figure 2). In addition, cross-linking of the etched dentin had no influence on the nanoleakage at the interfaces produced on wet dentin. Bonding adhesives to dry dentin (Figure 2) reduced silver nanoleakage of bonded interfaces (Figure 2). In these interfaces, the infiltration of silver ions was similar to that observed at the interfaces of the wet dentin specimens. An increase in the accumulation of silver was observed after 12 months of aging of the interfaces without biomodification of the etched dentin, especially at the interfaces produced on the dry dentin (Figure 3).

In Situ Zymography

The presence of interfacial fluorescence indicative of gelatinolytic activity was identified along the entire length of the interface of the nonbiomodified wet and dry dentin groups, both at the 24-hour (Figure 4) and 12-month (Figure 5) aging periods. The intensity and the linear area of fluorescence did not increase over

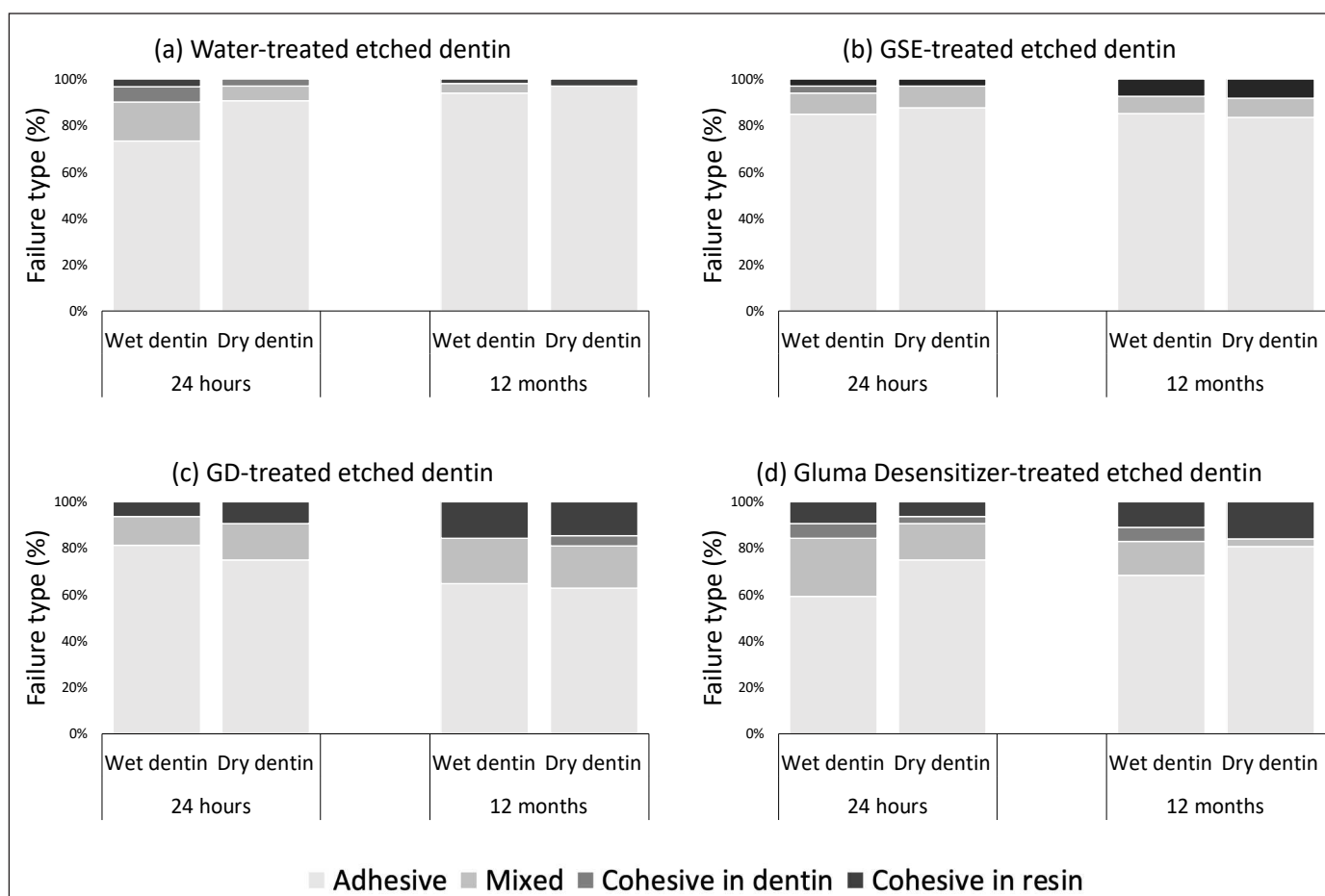


Figure 1. Distribution of types of failures of mechanically stressed bonded specimens stored in saliva-like buffer for 24 hours or 12 months. The adhesive system was applied to wet or air-dried etched dentin (a) with no biomodification (water-treated dentin) or previously biomodified with (b) 5% grape seed extract (GSE), (c) 5% glutaraldehyde (GD), or (d) Gluma Desensitizer.

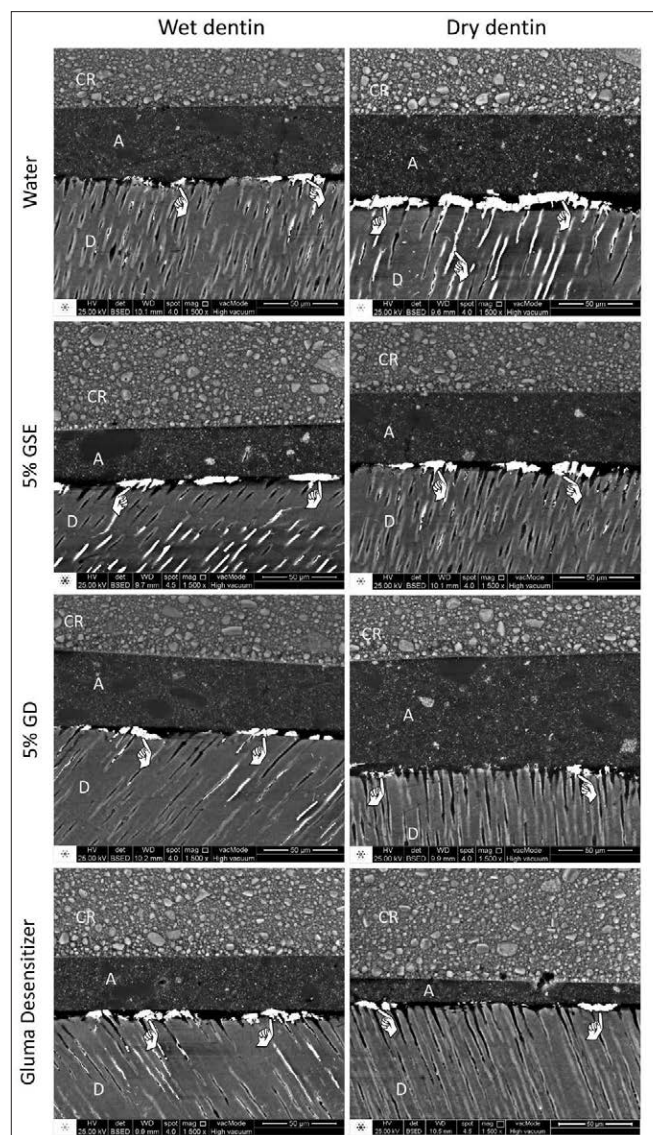


Figure 2. SEM (scanning electron microscopy) images of silver nanoleakage of resin-dentin bonds stored for 24 hours in saliva-like buffer. The two-step, etch-and-rinse adhesive was applied to wet or air-dried etched dentin previously biomodified with 5% grape seed extract (GSE), Gluma Desensitizer, or 5% glutaraldehyde (GD). A, adhesive layer; CR, composite resin; D, dentin; Pointers: silver nitrate accumulation areas. 500x.

time. Conversely, no or little fluorescence signal was detected in isolated areas of the interfaces produced on cross-linked dentin. Interfaces produced after the application of Gluma Desensitizer showed more fluorescence in both aging periods than interfaces produced after the use of GSE and GD.

DISCUSSION

The water-wet bonding technique⁴⁰ is still recommended when two- or three-step etch-and-rinse adhesive systems

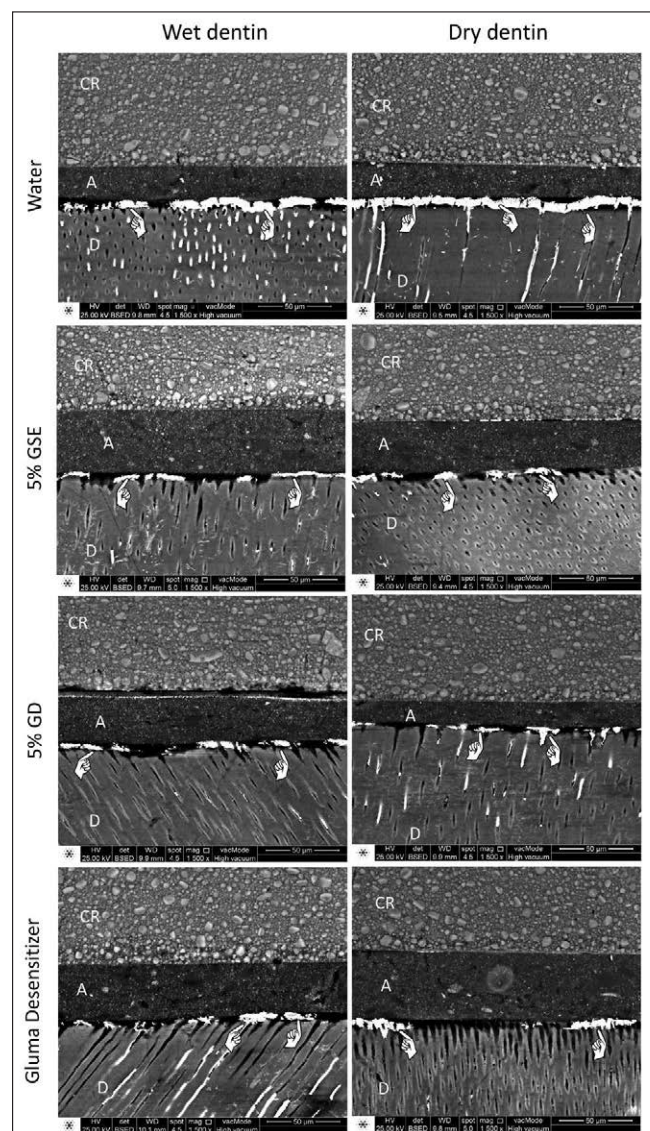


Figure 3. SEM (scanning electron microscopy) images of silver nanoleakage of resin-dentin bonds stored for 12 months in saliva-like buffer. The two-step etch-and-rinse adhesive was applied to wet or air-dried etched dentin previously biomodified with 5% grape seed extract (GSE), Gluma Desensitizer, or 5% glutaraldehyde (GD). A, adhesive layer; CR, composite resin; D, dentin; Pointers: silver nitrate accumulation areas. 500x.

are used. Despite the negative impact of the presence of residual water on nanoleakage⁴¹ and polymerization of adhesive monomers,^{19,20} water is important for the maintenance of interfibrillar collagen peptide spaces produced by the removal of hydroxylapatite (HA) nanocrystals by etching with phosphoric acid.¹⁸ The attempt to remove water simply by evaporation with air blasts resulted in a significant reduction of bond strength.²¹⁻²³ The same negative interference of drying etched dentin on the immediate bond strength was

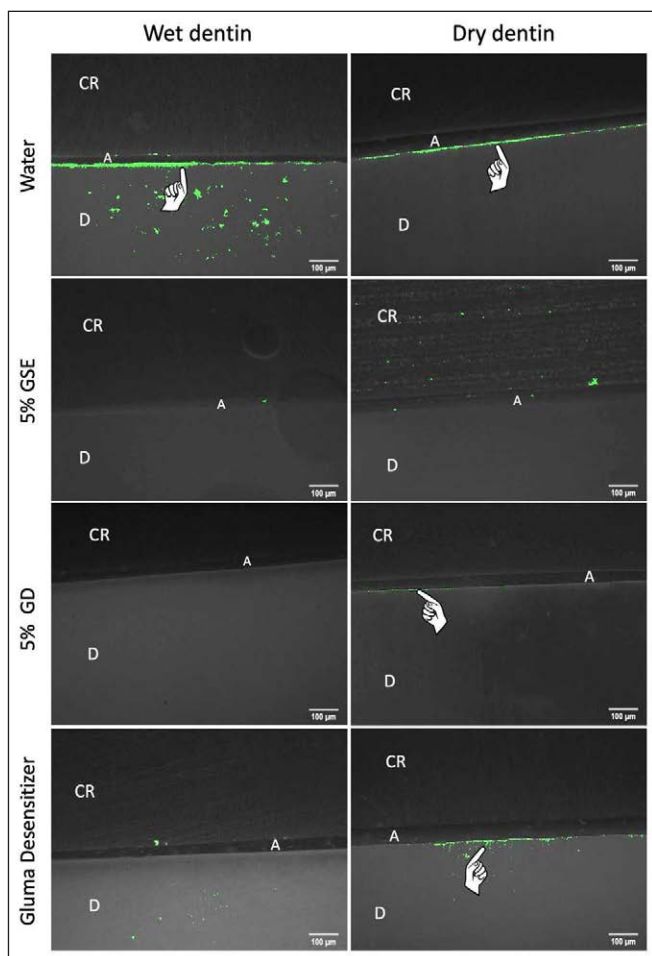


Figure 4. Fluorescence microscopy images of interfacial gelatinase activity of 24 hour-aged resin–dentin bonds. The two-step etch-and-rinse adhesive was applied to wet or air-dried etched dentin previously biomodified with 5% grape seed extract (GSE), Gluma Desensitizer or 5% glutaraldehyde (GD). Bright green fluorescence indicates collagenolytic activity. All images were captured using the same intensity, 10x. A, adhesive layer; CR, composite resin; D, dentin.

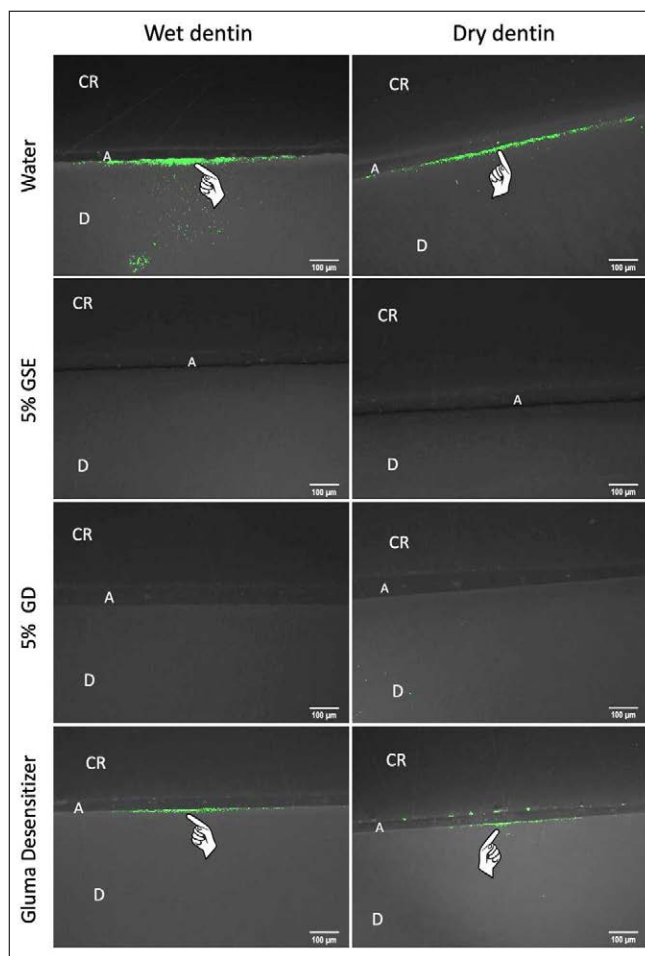


Figure 5. Fluorescence microscopy images of interfacial gelatinase activity of 12 month-aged resin–dentin bonds. The two-step etch-and-rinse adhesive was applied to wet or air-dried etched dentin previously biomodified with 5% grape seed extract (GSE), Gluma Desensitizer, or 5% glutaraldehyde (GD). Bright green fluorescence indicates collagenolytic activity. All images were captured using the same intensity, 10x. A, adhesive layer; CR, composite resin; D, dentin.

observed in the present study, with a reduction of 62% in the mean value in comparison to wet dentin. Additionally, interfaces produced on dry dentin showed higher nanoleakage uptake, demonstrating that low bond strength occurred when bonding resin was unable to envelop interfacial collagen.

After washing off the phosphoric acid used for demineralizing the dentin surface, the collagen fibrils depleted of their mineral reinforcement are maintained separated by the presence of water. In this state, the composition of dentin consists of approximately 30% collagen and 70% water, and the elastic modulus of the etched dentin matrix is only 3–5 MPa.^{18,26} Simply drying dentin surfaces with air results in collapse of the fibrils,²³ which obliterates the interfibrillar spaces.

The close spatial approximation of the collagen fibrils allows the spontaneous formation of hydrogen bonds between the peptides of neighboring fibrils⁴² resulting in the shrinkage of the dentin matrix.¹⁸ In addition to obliterating the interfibrillar spaces necessary for the infiltration of resin monomers, the stiffness of the dentinal matrix (Young's modulus) increases with drying (20–30 fold)⁴³ and may reach 170 MPa.^{44–46} Hydrogen bonds between dry collagen fibrils have a cohesive energy of $14.8 \text{ (J/cm}^3)^{1/2}$. To rupture such bonds, solvents must have a higher solubility parameter for hydrogen bonds (d_h). Water, for example, has a $d_h=40.4$ (Hoy's solubility parameter); this fact explains why water is capable of re-expanding dehydrated collagen matrices by 100%.¹⁸ Other solvents present in

the formulations of adhesive systems, such as acetone [$11(\text{J}/\text{cm}^3)^{1/2}$] and ethanol [$20(\text{J}/\text{cm}^3)^{1/2}$], have d_h that are lower or very close to $14.8^{18,47}$ —the strength of hydrogen bonds between adjacent collapsed collagen peptides in air-dried etched dentin.

The adhesive Optibond S used in this study contains ethanol as its solvent. A water-free adhesive was selected to prevent this solvent from interfering with the wetness of the dried dentin. Bond strengths of One-Step (Bisco Inc, Schaumburg, IL, USA), which is an acetone-based adhesive, were negatively affected when applied on air-dried, etched dentin, while a tertiary, butanol-based organic solvent adhesive XP Bond (Dentsply Sirona, Milford, DE, USA) performed equally well in both the wet- and dry-bonding techniques.⁴⁸ However, even the application of an adhesive containing ethanol and water as solvents was not capable of countering the negative effects of drying the etched dentin with air.²³ Ethanol has a solubility parameter d_h [$20(\text{J}/\text{cm}^3)^{1/2}$] close to the value necessary for rupturing the hydrogen bonds established between dry collagen fibrils [$14.8(\text{J}/\text{cm}^3)^{1/2}$].^{18,47} Therefore, the capacity of ethanol to re-expand collapsed dentin matrices is limited. The dehydrated acid-etched matrix and its subsequent saturation with ethanol has a thickness of only 30% of that observed for the matrix maintained saturated with water.⁴⁷ Other components of the adhesive such as bisphenol A-glycidyl methacrylate (Bis-GMA) ($d_h=5.8$) and hydroxyethyl methacrylate (HEMA) ($d_h=15.2$) are incapable of re-expanding the collapsed matrix.¹⁸ In addition to reducing the immediate bond strength value by 62% in comparison with the bond to wet dentin, interfaces produced by the application of adhesive on dentin dried with air were unstable in the long term. After 12 months of aging, the mean bond strength of these interfaces was 37% lower than the immediate bond strength value. The intense uptake of silver observed at these interfaces indicated solubilization of collagen peptides in the hybrid layer in this period and their replacement by water.

The increase in the number of intramolecular, intermolecular, and interfibrillar cross-links promoted by the application of cross-linkers improved the mechanical properties of collagen and, consequently, of the tissues with a high content of this protein.^{25,26,28,29} The negative effects promoted by excessive drying of the etched dentin were prevented by the cross-linking of collagen with an extract rich in proanthocyanidins (GSE) prior to the application of the adhesive system.²³ In the same way, all the cross-linkers investigated in the present study were capable of making the collagen more resistant to the point where drying of the biomodified matrix did not have any negative effects

on the immediate bond strength. Also, the amount of silver uptake detected became similar to those at the interfaces established on wet dentin, as seen in the SEM images. The bond strength values to dry dentin, when dentin was previously biomodified with GSE, Gluma Desensitizer, or GD, were comparable with the values obtained for dentin that was kept wet. For this condition of hydration, none of the cross-linkers affected the immediate bond strength; that is, the values of all the groups were comparable with those of the wet control without biomodification and similar among them.

After 12 months of aging in a saliva-like buffer, a significant drop in bond strength was seen for interfaces produced on nonbiomodified etched dentin for both wet- and dry-bonding techniques. The overall reduction in bond strength was 37%. One would expect a higher reduction in bond strength for interfaces created on dry dentin, with no previous biomodification of the collagen. Since a similar reduction was seen, it could be assumed that the etched dentin had a lower water content that favored the infiltration of more hydrophobic monomers into the interfibrillar spaces. This may have minimized phase separations¹⁸ and improved monomeric conversion, making the resinous content of the hybrid layer more resistant to degradation. Conversely, bonds produced on wet and dry biomodified dentin remained mechanically stable after 12 months of aging.

Hybrid layers can deteriorate when the resin component is solubilized by esterases,⁴⁹ and collagen is solubilized by endogenous MMPs and cysteine cathepsins.⁵⁰ These enzymes are hydrolases that use water to break chemical bonds.^{16,51,52} Thus, air drying the etched dentin could impair the activity of such enzymes by limiting their access to water, thereby reducing the hydrolytic activity within the hybrid layer. However, the intense collagenolytic activity detected in the interfaces created on dry-etched dentin with no previous biomodification refutes that hypothesis. Most water molecules in the acid-etched dentin are found in a free form (75%-79%), while 21%-25% is bound to collagen and unlikely to be removed.¹⁰ Therefore, even after 60 seconds of air drying, the residual water in the etched dentin was enough to enable the activity of the dentin proteases.

The biomodification of dentin collagen by the use of cross-linkers reduces its enzymatic breakdown. MMPs are unable to cleave the tightly wound triple helix of collagen, because the opening of their catalytic site is smaller (~ 0.5 nm) than the diameter of the collagen molecule (~ 1.5 nm).^{16,53} To degrade the collagen molecule, MMPs need to unwind or separate each collagen polypeptide chain and cleave each peptide individually. The increased number of secondary

cross-links produced by cross-linkers makes it difficult to separate the chains. Additionally, despite increasing collagen stiffness, cross-linkers also increase the number of secondary cross-links within MMPs and cysteine cathepsins, impairing the exposure and docking of the catalytic site to the substrate receptor.¹⁶ The new cross-links seem to be stable longitudinally, even when the hybrid layer is exposed to exogenous proteases from *Clostridium histolyticum*.³⁰ Proanthocyanidins are more efficient in inhibiting matrix metalloproteinases (MMP-2, MMP-8, and MMP-9) and cysteine cathepsins (CAT B and CAT K) than is 2% chlorhexidine.³¹ The same superiority was seen for 5% glutaraldehyde against MMPs.³²

Gluma Desensitizer (Kulzer GmbH, Hanau, Germany) is a dentin desensitizer that has been on the market for over 20 years. It is an aqueous solution of 5 wt% glutaraldehyde and 35% HEMA.⁵⁴ Its mechanism to treat dentin hypersensitivity is the coagulation of soluble plasma proteins in exposed dentinal tubules. The coagulated proteins in dentinal tubules reduce hydrodynamic fluid movement.⁵⁵ The MMPs' inhibitory capacity of Gluma Desensitizer is equivalent to that of 5% glutaraldehyde in water, showing that the presence of HEMA does not affect the antienzymatic effect of glutaraldehyde.^{32,54} The use of a 5% glutaraldehyde solution has been shown previously to be effective in producing mechanically stable hybrid layers.²⁸ In the present study, the application of Gluma Desensitizer on etched dentin allowed air drying with no detrimental effects on the immediate bond strength. Additionally, bonds produced on Gluma Desensitizer-modified dentin were mechanically stable after 12 months of aging, and showed minimal nanoleakage and proteolytic activity.

In situ zymography using fluorescence or laser scanning *confocal* microscopy is a well-known technique used to identify gelatinolytic activity in biological tissues. This technique has been used to demonstrate the proteolytic activity in both mineralized and demineralized dentin,⁵⁶ and in resin–dentin interfaces.^{57–59} Following the demineralization of dentin, intense proteolytic activity was detected in the hybrid layer produced on the nonbiomodified dentin. It has been shown that after the intense initial activity of dentin proteases, there is a progressive reduction in the release of ICTP (carboxyterminal telopeptide of type I collagen) and CTX (C-terminal telopeptides of type I collagen)—biomarkers of type I collagen degradation by MMPs and cysteine cathepsins, respectively,⁶⁰ suggesting that these proteases are able to degrade only the substrate within the reach of their molecular mobility. The detection of fluorescence within the

hybrid layers after 12 months of aging seems to challenge that statement. Conversely, no or minimal proteolytic activity was seen in aged interfaces produced after the biomodification of the dentin collagen, especially with GSE and GD. The long-term effect of dentin collagen biomodification by proanthocyanidins was also demonstrated by the sustained increased mechanical stability and resistance to degradation by endogenous proteases and bacterial collagenase after 18 months.³⁰

CONCLUSIONS

Bonded resin–dentin interfaces produced using the cross-linked, dry bonding technique were as mechanically stable as the interfaces produced using cross-linkers with the current recommended water-wet bonding technique. Nanoleakage was also reduced in biomodified dentin bonds for up to 1 year of aging. As proteolytic activity in specimens treated with cross-linkers in both adhesive techniques was also similar, therefore, all the null hypotheses were accepted. The cross-linking of the etched dentin not only improved the stability of hybrid layers against degradation but also eliminated the sensitivity of the adhesion techniques as to the degree of dentin wetness, even if a water-free adhesive system is used. However, the number of steps and the time necessary for the cross-linked dry bonding technique is much greater, especially if compared with the application of a universal adhesive, even if performing the selective acid etching of enamel with phosphoric acid. The effectiveness of the cross-linked dry bonding technique still needs to be investigated in well-conducted randomized clinical trials. The use of such a multi-step technique should be considered only if the clinical performance over time is proven to be superior to the conventional wet-bonding approach when using etch-and-rinse adhesive systems.

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Regulatory Statement

The author represents that the study was performed in compliance with author's institution's appropriate policies related to the use of animal and/or human subjects and human-derived material.

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

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In Vitro Biocompatibility of CPP-ACP and Fluoride-containing Desensitizers on Human Gingival Cells

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Clinical Relevance

Different commercial desensitizers with the same therapeutic indication exhibit different biological effects and cytotoxicity on human gingival fibroblasts, which could be considered criteria for choosing the optimal treatment of dentin hypersensitivity.

SUMMARY

Objectives: To analyze the biocompatibility of different desensitizers containing casein phosphopeptide-amorphous calcium phosphate

(CPP-ACP) and fluoride in their composition: MI Varnish (MV), Clinpro White Varnish (3M Oral Care), Profluorid Varnish (VOCO), Duraphat (Colgate) and Embrace Varnish (Pulpdent) on human gingival fibroblast cells (hGF).

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Methods and Materials: Human gingival fibroblast (hGF) cells were exposed to several desensitizer extracts at different concentrations (0.1%, 1%, and 4% eluates). Then, *in vitro* biocompatibility was studied by analyzing the IC_{50} value, cell proliferation (MTT assay and cell cycle), cell migration (wound healing assay), cell morphology and F-actin content (immunocytofluorescence), and induction of apoptosis/necrosis (flow cytometry). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test.

Results: The lowest cell viability and IC_{50} were observed in all concentrations of Embrace Varnish-treated hGFs ($p < 0.001$), whereas the highest were exhibited by those treated with Clinpro White Varnish. Similar effects were evidenced when induction of apoptosis/necrosis and cell migration assays were assessed. Finally, MI Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish extracts showed lower numbers of attached cells, some of them with an unusual fibroblastic morphology when cultured with 4% concentration of the varnishes, while Clinpro White Varnish exhibited a similar number of cells with an evident actin cytoskeleton compared to the control group.

Conclusions: The results obtained in this study indicate that hGFs show better *in vitro* biocompatibility after exposure to Clinpro White Varnish, even at the highest concentration employed, making it the most eligible for topical applications. In contrast, Embrace Varnish exhibited a high cytotoxicity towards hGFs that could potentially delay the healing process and regeneration of the oral mucosa, although more studies are needed to confirm this hypothesis.

INTRODUCTION

The average prevalence of dentin hypersensitivity (DH) is 33.5%,¹ affecting the quality of life of a high number of patients by making it difficult for them to perform simple tasks such as drinking cold water, eating ice cream, or even brushing their teeth.^{2,3}

The pathogenesis of DH has been explained by different theories throughout history until 1964, when the hydrodynamic theory proposed by Brännström was established, which to date is the most widely accepted theory.⁴ Previous reports showed that the DH process is complex and multifactorial, requiring the presence of gingival recession and/or cementum loss from root surfaces that is usually caused by periodontal disease,

although it can also be favored by erosion, attrition, or abrasion.^{5,6} Once this happens, it is easier for a stimulus, either thermal, tactile, osmotic, or chemical dehydration, to produce an acute and brief pain sensation.^{7,8}

Some researchers have shown it is necessary to target pulpal nerves directly to reduce DH or to occlude the dentinal tubes with a precipitating substance or a sealing agent.^{9,10} Thus, various treatments for DH include the use of lasers, biocrystals, fluorides, adhesives, oxalates, arginine, or potassium salts.^{5,9}

Among all these eligible treatments, one of the most frequently employed in clinical practice, due to their low cost, are the fluoride varnishes.¹⁰⁻¹² These varnishes are applied onto the dental surface, and the chemical components that form are released throughout the oral cavity. They can be detected in the saliva or adhered to the tooth surface (which will cause it to continue releasing over time), and also penetrate into dentinal tubules.¹³ For these reasons it is important to assess the toxicity these products may produce in the oral resident fibroblasts with which they come into contact when they diffuse through the gingival tissue. Although there are studies that assess the toxicity of toothpastes for tooth sensitivity,¹⁴ or other products commonly used for DH treatment,¹⁵ there are not many studies about toxicity of fluoride varnishes indicated for DH.^{15,16}

Recently, a dental varnish (MI Varnish, GC, Tokyo, Japan) containing fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) was introduced in the market,¹⁷ and Clinpro White Varnish (3M Oral Care, North Ryde, Australia) contains tricalcium phosphate. These varnishes include bioactive materials that have a desensitizing action when applied onto the tooth surface.¹⁸ These bioactive agents are popular due to their biocompatibility and similar crystal structure to the dental tissues.¹⁹⁻²¹ Other fluoride varnishes such as Profluorid (VOCO, Cuxhaven, Germany) and Duraphat (Colgate Palmolive, Hamburg, Germany) do not have new formulas in their composition, being more traditional varnishes, with the exception of Embrace Varnish (Pulpdent, Watertown, MA) that contains xylitol—a polyalcohol that also acts as caries preventive agent.²² However, the number of published articles evaluating the biocompatibility of these products is scarce, and only one study on Clinpro White Varnish biocompatibility has been previously reported, which concluded it presents less cytotoxicity than the other products for DH.¹⁵ In particular, the tests with fluoride- or calcium phosphate-based desensitizers are practically null in the literature, which was another reason for this study.

In vitro toxicity tests are useful tools to evaluate the biocompatibility of different materials and are very

frequently used in a wide variety of studies.²³⁻²⁶ Thus, different oral cell culture models are commonly used to elucidate the mechanisms involved in different biological responses and to investigate oral cell behavior in specific situations.²⁷

The aim of this study was to compare the biocompatibility and cytotoxicity of several desensitizers employed in daily clinical practice for the treatment of dental hypersensitivity such as MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish on human gingival fibroblasts (hGF). The null hypothesis tested was that there are no significant differences among the different desensitizers in terms of their cytocompatibility.

METHODS AND MATERIALS

Preparation of Desensitizer Eluates

The tested materials included five commercially available desensitizers: MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish. Manufacturer's data, composition, and lot number of each of the tested materials are shown in Table 1. Eluates of these materials were prepared following the recommendations of ISO 10993-5.²⁸ To obtain a 10% concentration, 1 ml of each fluoride varnish was mixed gently with 9 ml of Dulbecco's Modified Eagle's Medium (DMEM) culture medium (Gibco, Thermo Fisher Scientific, Carlsbad, CA, United States) and filtered through a 0.22- μ m syringe filter. Then, eluates were subsequently diluted with culture medium to obtain different dilutions (0.1%, 1%, and 4%) and sterilized by exposure to ultraviolet light for 2 hours. DMEM culture medium without any eluates served as a negative control.

Isolation and Culture of Human Gingival Fibroblasts

Human gingival fibroblasts (hGFs) were obtained from impacted wisdom teeth (n=8). All participating donors previously signed an informed consent form according to the Helsinki Declaration guidelines.

After extraction, gingival tissues were extensively rinsed with phosphate-buffered saline (PBS) and cut into 1-mm³ tissue blocks after removing blood vessels. Then, the tissue blocks were placed on polystyrene tissue culture flasks and suspended in PBS containing 1% penicillin/streptomycin (Invitrogen, Paisley, Scotland) to avoid oral bacterial contamination, and digested with serum-free DMEM culture medium containing 0.1% collagenase A (Roche Diagnostics, Basel, Switzerland) and 0.2% dispase II (Sigma-Aldrich, St. Louis, MO, United States) for 2 hours at 37°C. Then, isolated hGFs were washed with PBS, filtered through 40-mm nylon cell strainers (BD Biosciences, San Jose, CA, United States) and cultured in DMEM culture medium supplemented with 10% fetal calf serum (Lonza, Basel, Switzerland), 1% GlutaMAX (ThermoFisher Scientific), and 1% penicillin/streptomycin (ie, complete growth medium) at 37°C and 5% CO₂. For subsequent experiments, hGFs were used from culture passage 2 up to 6.

MTT and IC₅₀ Assays

For cytotoxicity evaluation, 1×10⁴ hGFs were cultured in the presence of the five studied desensitizer eluates or in DMEM culture medium alone (negative control) prepared as before. Cell metabolic activity was measured 24 hours, 48 hours, and 72 hours after the beginning of the cultures by MTT assay. According to the manufacturer's instructions, the MTT reagent (Sigma-Aldrich) was added to the wells for 4 hours. When the purple precipitate was obviously noticeable, dimethylsulfoxide

Table 1: *Tested Materials*

Material	Manufacturer	Composition	Lot Number
MI Varnish	GC Corporation	5% NaF (22.6 mg) + Casein Phosphopeptide-Amorphous Calcium Phosphate	1810041
Clinpro White Varnish	3M Oral Care	5% NaF (22.6 mg) + Tricalcium Phosphate	N994659
Profluorid Varnish	VOCO	5% NaF (22.6 mg), ethyl acetate-isoamylpropionate, polyurethane, difluorosilane	1931557
Duraphat	Colgate Palmolive	5% NaF (22.6 mg)	213549
Embrace Varnish	Pulpdent	5% fluoride (22.6 mg) + Xylitol-coated Calcium and Phosphate (CXP)	190314

(DMSO) (Sigma-Aldrich) (100 μ l/well) was added to solubilize the formazan dye. Covered plates were kept in the dark for 2-4 hours. Finally, absorbance at 570 nm wavelength in each well was measured in a microplate reader (ELx800; Bio-Tek Instruments, Winooski, VT, United States). Each experimental condition was performed in quintuplicate for each desensitizer and analyzed in three independent experiments.

Complementarily, the dose of each of the tested materials that could decrease cell viability by 50% after 72 hours of culture (ie, IC_{50}) was analyzed graphically by plotting the percentage of the metabolic activity obtained in previous MTT assays on the Y axis and the percentage concentration of each fluoride varnish on the X axis. IC_{50} data were analyzed by nonlinear regression using GraphPad Prism software (version 8.1.0, GraphPad Software Inc, San Diego, CA, USA).

Cell Cycle Analysis

Cell cycle analysis by measuring the DNA content is a method that most frequently employs flow cytometry to distinguish cells in different phases of the cell cycle. Cells were cultured in 25 cm^2 culture flasks at 3×10^5 cells/ cm^2 in presence of the five studied desensitizers' extracts prepared at different concentrations (0.1%, 1%, and 4%) as described above for 24 hours. Then, 1×10^5 cells were collected, fixed in 70% ethanol, and incubated with 40 μ g/ml of propidium iodide and 200 μ g/ml RNase for DNA content analysis. Propidium iodide fluorescence was measured with a FACSCanto II Flow Cytometer (Becton Dickinson, San Jose, CA) excitation wavelength: 488 nm; emission wavelength: 617 nm) and the percentage of cells in G_0/G_1 , S and G_2/M phases was analyzed using CellQuest and Modfit LT programs (Becton Dickinson). Each experimental condition was performed in triplicate for each desensitizer and analyzed in three independent experiments.

Cell Migration

A wound healing assay was used to determine hGF migratory ability in presence of the five studied desensitizers' eluates. 2×10^5 hGFs/well were plated in 6-well plates to create a confluent monolayer. Then, the monolayer was scraped in a straight line to create a scratch or wound with a 100 μ l pipette tip, washed twice with PBS to remove detached cells, cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the five tested desensitizers prepared as before and imaged using a phase-contrast microscope at 0, 24, 48, and 72 hours. The open wound area between both fronts of cell migration was quantified using Image J

software (National Institutes of Health, Bethesda, MD, United States). Data are represented as percentages of migrated cells related to open wound areas just after scratching (100% of open wound area) and expressed as the mean $\pm$ standard deviation (SD) from three independent experiments performed in triplicate for each desensitizer.

Cell Cytoskeleton Staining

Phalloidin staining was used to analyze possible changes in cell morphology, and in the actin cytoskeleton structure and organization of hGFs cultured with the five studied eluates or DMEM culture medium alone as a negative control. Briefly, 3×10^4 hGFs were seeded on glass coverslips, allowed to adhere, and cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the five studied desensitizers' eluates for 72 hours from cell seeding at 37°C. Then, hGFs were rinsed twice with prewarmed PBS at 37°C, fixed in 4% formaldehyde solution (Merck Millipore, Darmstadt, Germany) for 10 minutes, permeabilized with 0.25% Triton X-100 solution (Sigma-Aldrich) for 5 minutes, and rinsed thrice with PBS. Cell cytoskeleton and nuclei were then stained at room temperature in the dark for 30 minutes with Invitrogen AlexaFluor594-conjugated phalloidin (ThermoFisher Scientific) and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (ThermoFisher Scientific), respectively. Finally, immunofluorescence images were observed in a Leica TCS SP2 confocal microscope (Leica, Wetzlar, Germany). Each experimental condition was carried out in triplicate for each desensitizer and analyzed in three independent experiments.

Apoptosis/Necrosis Assay

To evaluate hGF viability after exposure to the different desensitizers, 1×10^5 hGFs per experimental condition were cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the different eluates for 72 hours from cell seeding at 37°C. Cell viability was assessed by incubation with 5 ml of Annexin-V-FITC and 5 ml of 7-AAD staining (BD Biosciences) in 100 μ l 1 $\times$ Annexin-V buffer per experimental condition in the dark for 15 minutes at room temperature. Samples were analyzed in an LSR Fortessa X-20 flow cytometer (Becton Dickinson) within 1 hour of staining. Finally, the percentages of viable (double negative), early apoptotic (Annexin-V-FITC positive, 7AAD negative), and late apoptotic and necrotic (double positive and Annexin-V-FITC negative/7-AAD+, respectively) cells were determined. Each experimental condition

was performed in triplicate for each desensitizer and analyzed in three independent experiments.

Statistical Analysis

Migration and MTT data were represented as the mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) or Student's *t*-test followed by Tukey post hoc comparison test was used for statistical comparison among groups using GraphPad Prism software version 8.1.0 (GraphPad Software, Inc). *p*-values < 0.05 were considered significant.

RESULTS

IC₅₀ and MTT Assays

Viability of hGF in contact with the desensitizer extracts varied depending on the culture time, concentration, and material used (Figure 1). At 0.1% concentration, only Embrace showed a slight but significant decreased cell viability at all times studied compared to the control group (*p*<0.001), whereas at 1% concentration the comparison of cell viability among the different desensitizers and control group was: Control > Clinpro White Varnish > MI Varnish > Profluorid Varnish > Duraphat > Embrace (*p*<0.001). Finally, at 4% concentration, cell viability was more compromised at all times than that observed at 1% and ordered from highest to lowest as: Control > Clinpro White Varnish > Profluorid Varnish = Duraphat > MI Varnish = Embrace (*p*<0.001). Given these results, the IC₅₀ values at 72 hours of culture (ie, percentage

concentration of each desensitizer to inhibit 50% of hGF viability) were: Clinpro White Varnish = 4.4%; Profluorid Varnish = 1.6%; Duraphat = 1.4%; MI Varnish = 1.1%, and Embrace = 0.2% (Figure 2).

Cell Cycle Analysis

Cell cycle phase distributions are shown in Figure 3. At 4% concentration, in the Clinpro White Varnish group, the majority of the cells were found in G₀/G₁ phase (73.40%) with very few cells in S phase (15.88%) and G₂/M phase (10.72%). Conversely, MI Varnish, Duraphat, and Profluorid showed that the percentage of cells in phase G₀/G₁, S and G₂/M was 44.79%-50.59%, 26.75%-39.09%, and 16.77%-22.66%, respectively. At 1% concentration, only Embrace exhibited few cells in G₀/G₁ phase (50.24%); whereas, at 0.1% concentration, all groups showed abundant cells in G₀/G₁ phase (74%-76%).

Cell Migration

Open wound areas of migrating hGFs in presence of different concentrations of the analyzed desensitizers were measured after 24, 48, and 72 hours after wound infliction of confluent hGF monolayers (Figure 4). In general, except with Clinpro White, migration rates exhibited by hGFs cultured with any of the tested fluoride varnishes at 4% concentration were significantly lower at 24, 48, and 72 hours compared to the control group (*p*<0.001). At 1% concentration, Embrace displayed a statistically significant decreased cell migration after 24,

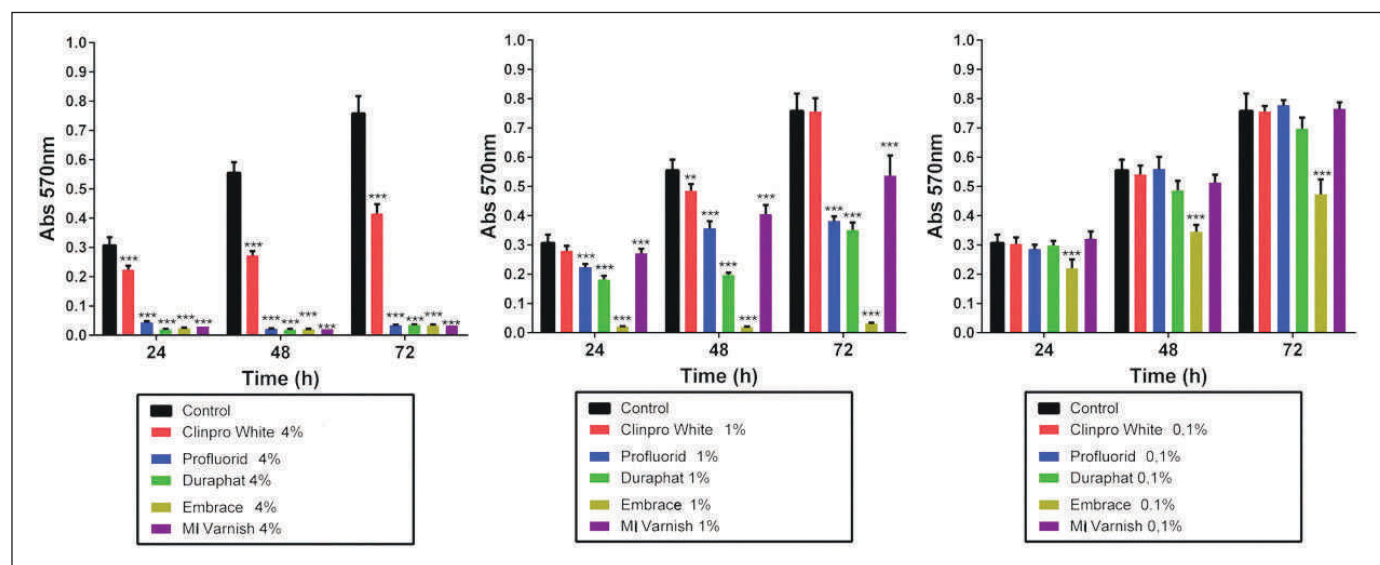


Figure 1. Analysis of the metabolic activity of human gingival fibroblasts (hGFs) after exposure to different concentrations of desensitizer varnishes by MTT assays. Absorbance at 570 nm was significantly lower compared to the control conditions (***p*<0.01; ****p*<0.001, respectively) by one-way analysis ANOVA followed by Tukey post hoc test. Data are expressed as mean $\pm$ SD from *n* = 3 separate experiments.

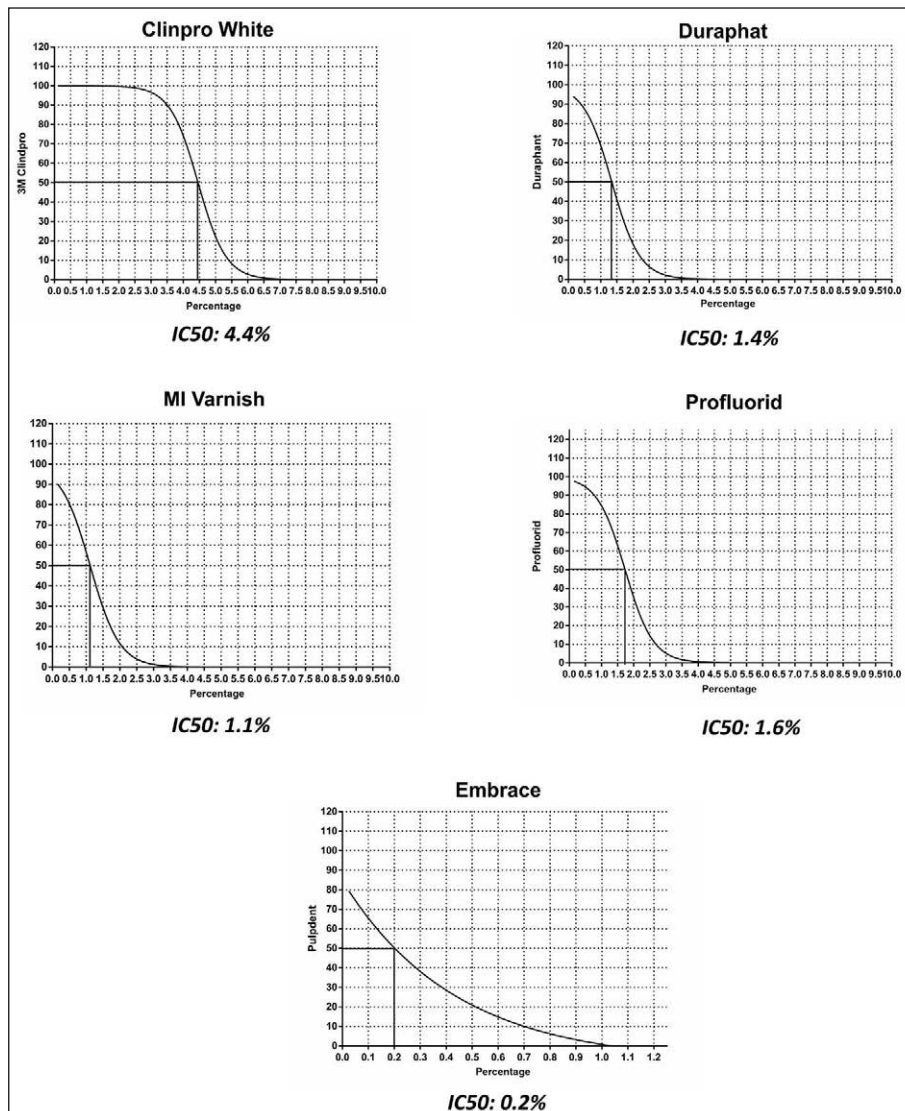


Figure 2. Calculation of IC₅₀ values of the different desensitizers. The percentage concentration of each varnish in the extract required for a 50% inhibition of metabolic activity of hGFs after 72 hours of culture was calculated. Data were analyzed by nonlinear regression by plotting the percentage of the metabolic activity on the Y axis and the percentage of each varnish on the X axis. Curves shown are representative from $n = 3$ separate experiments.

48, and 72 hours of culture ($p < 0.001$), while no significant differences were found among the other sensitizer groups. Finally, at 0.1% concentration, neither Clinpro White, Duraphat, MI Varnish, Profluorid, nor Embrace showed a significant decrease in the hGF migration compared to the control at any of the times studied.

Cell Cytoskeleton Staining

After 72 hours of exposure of hGF cultures to the five sensitizer extracts at 0.1% concentration, many well-attached and spread cells with a fibroblastic spindle-shaped morphology and high F-actin content were observed, similar to the control cells. However, Embrace at 1% concentration showed a considerably smaller number of attached cells with an aberrant morphology, whereas the other sensitizer groups exhibited a high number of well-adhered cells. Finally, Duraphat, MI

Varnish, Profluorid, and Embrace extracts showed smaller numbers of attached cells, some of them with an aberrant morphology at 4% concentration, while Clinpro White exhibited a similar number of cells with well evident actin cytoskeleton compared to the control group (Figure 5).

Apoptosis/Necrosis Assay

The apoptosis/necrosis rate in each group was calculated by flow cytometry. As shown in Figure 6, at 4% of the concentration the percentage of viable cells was: Clinpro White (99.1%) > MI Varnish (98.03%) > Profluorid (90.9%) > Duraphat (11.7%) > Embrace (5.79%), whereas early/late apoptotic and necrotic cell percentages were: Clinpro White (0.9%) < MI Varnish (1.97%) < Profluorid (9.1%) < Duraphat (88.3%) < Embrace (94.21%). At 1% concentration, all materials

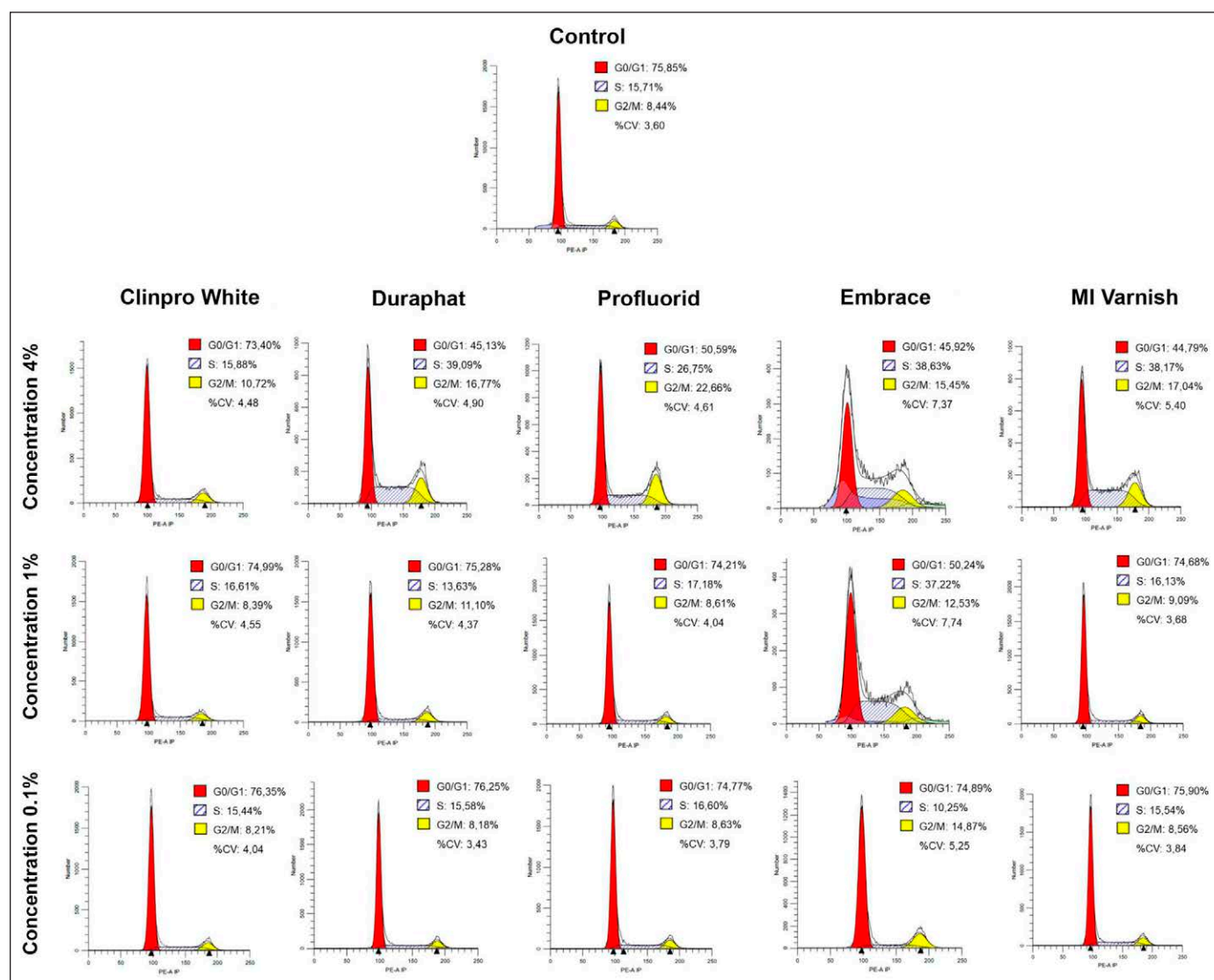


Figure 3. Cell cycle analysis of the human gingival fibroblasts (hGFs) after exposure to the desensitizer concentrations (0.1%, 1%, and 4%). Dot-plots shown are representative from $n = 3$ separate experiments.

displayed a high percentage of viable cells (>95%), except with Embrace (7.83%). Similarly, at 0.1% concentration, all desensitizers displayed a similar biocompatibility compared to the control medium, exhibiting more than 95% of viable cells, except with Embrace (88.3%).

DISCUSSION

Bioactive dental products were recently introduced and developed to optimize properties such as ion release, the promotion of remineralization, or the stimulation of hydroxyapatite formation.^{29,30} Thus, varnish desensitizers containing fluoride (F), calcium (C), phosphate (P), or CPP-ACP such as MI Varnish, Clinpro White [fluoride (F), calcium (Ca²⁺), phosphate PO₄³⁻], or Embrace, have been commercially released.

The results of this study showed that there are statistically significant differences among the different desensitizers in terms of their cytocompatibility. Hence, the null hypothesis was rejected. There is a lack of studies that assesses the toxicity of varnish desensitizers among the available literature. These products have been used for years, when biocompatibility was not yet taken into account, so it has been assumed that there are no biocompatibility problems with them; but, as we have shown in this study, there are differences between some products with regard to biocompatibility, so it is an important issue to investigate before continuing with their clinical use. Eyüboğlu and others¹⁵ evaluated the cytotoxicity of dental cells after contact with dentin-desensitizing products (not only varnish desensitizers).

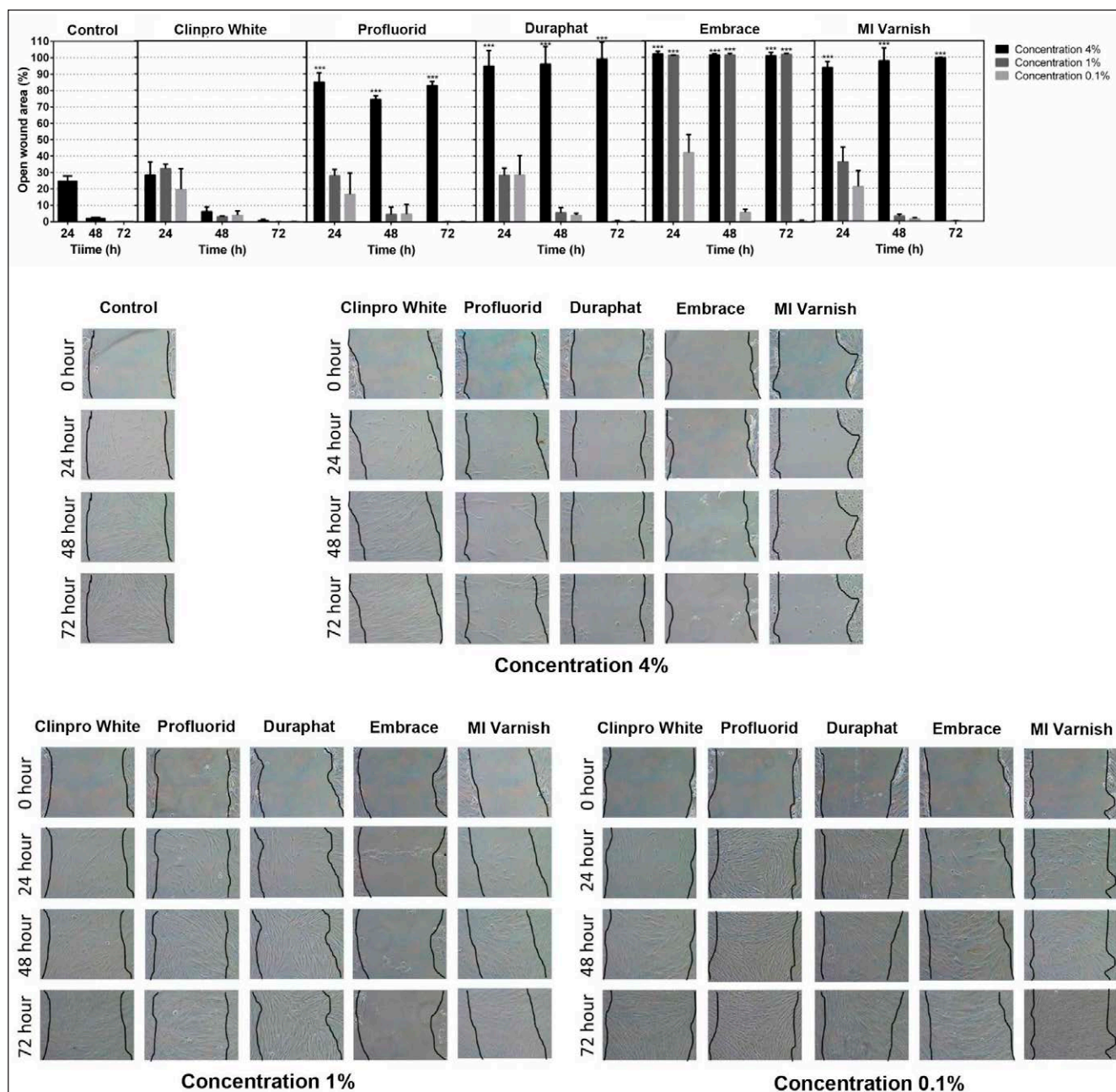


Figure 4. Cell migration was evaluated using wound healing assays. Cells were cultured at different concentrations of the different desensitizers. The control condition consisted of cells cultured in culture growth medium without any desensitizer. Open wound areas were measured at 24, 48, and 72 hours and compared to the initial scratched wound area at 0 hour in the same well (100% migration). Cell migration was significantly lower compared to the control conditions (* $p < 0.05$; *** $p < 0.001$, respectively) by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Data are expressed as mean $\pm$ SD from $n = 3$ separate experiments.

and noticed that some of them were cytotoxic towards human gingival cells and pulp fibroblasts. In this study we used human gingival cells, since this is the only population of cells exposed to the application of the varnish. Other authors have also previously tested fluoride varnishes using hGFs or other fibroblast cell lines to evaluate DH and cytotoxicity.^{16,31,32}

The use of MTT, IC_{50} , cell cycle analysis, cell migration, cell cytoskeleton staining, and apoptosis/necrosis assays helps us to assess the biological behavior of human fibroblasts when they are exposed to these commercial desensitizers, showing an altered cell morphology or adhesion, a greater level of apoptotic and necrotic cells, and lower proliferation with some of these products.

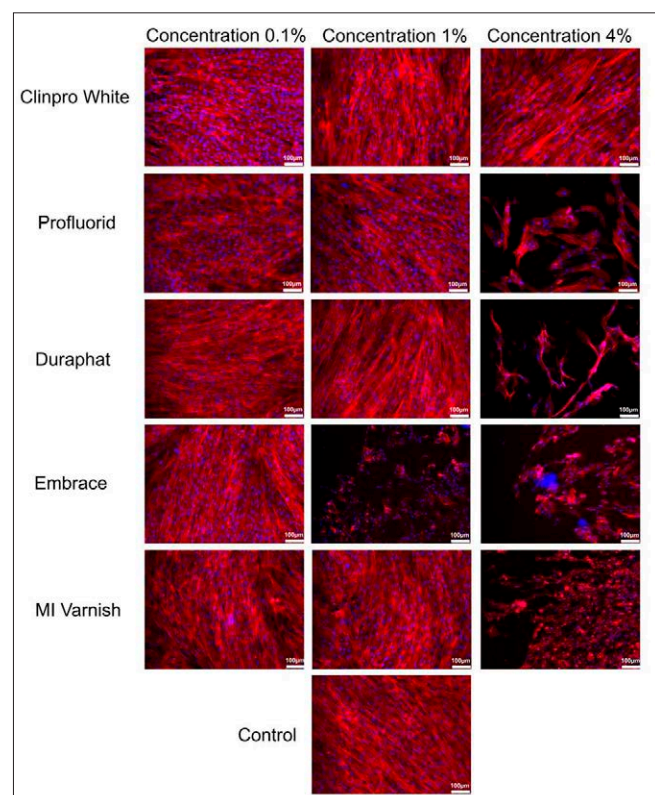


Figure 5. Analysis of cell morphology changes in the actin cytoskeleton structure and organization on hGFs after treatment with the different desensitizers by confocal fluorescence microscopy. F-actin fibers were stained with AlexaFluor 594-conjugated phalloidin (red), whereas cell nuclei were counterstained with DAPI (blue). Confocal fluorescence microscopy images shown are representative from $n = 3$ separate experiments. Scale bar: 100 micron.

In the present study, the MTT assays revealed that Embrace negatively affected human gingival cell viability at all extraction medium concentrations, while Clinpro White and MI Varnish showed cytotoxicity, which decreased with further dilutions. At the same time, these products exhibited IC_{50} values of 4.4% and 1.1%, respectively. In a similar manner, a previous study reported that Clinpro White showed less cytotoxicity on gingival and dental pulp cells than other desensitizing products.¹⁵ In addition, it has been reported that Duraphat in intimate contact with the oral tissues is not potentially harmful to host cells.³¹ In the present study, we observed cytotoxicity at 4% and 1% but not at 0.1% concentrations.

It is well known that desensitizers release substances that could potentially delay or improve healing.³³ For this reason, we decided to use wound healing assays in order to preliminarily predict how the coordinated migration of hGFs would occur during inflammation or after injury. The marked decrease in cell migration in the Embrace-treated group could be due to the effect

of these materials on cell viability. Our cell migration results suggest a correlation with our cell attachment and spreading results. In general, cells need to attach and spread on a surface for subsequent migration. Low cell attachment and F-actin fiber content and an aberrant morphology were evidenced in the 4% and 1% Embrace-treated groups, as observed by phalloidin staining; hence, their migration would also be affected. Although all materials studied presented 5% sodium fluoride in their composition, other components could be responsible for their biological effects on hGFs. Among the limitations of the present study could be the lack of experiments to assess the ions released by the different desensitizers to determine what composition can alter their biocompatibility. In fact, previous studies reported that low levels of sodium fluoride promote cell proliferation, cell migration, and accelerates wound closure, in turn.^{34,35}

Apoptosis/necrosis assay evidenced a reduced number of viable cells in presence of Embrace varnish. A previous study reported that high concentrations of sodium fluoride (5000 ppm) promoted apoptotic morphological changes and DNA fragmentation on cementoblasts.³⁶ However, our observations suggest that sodium fluoride may not be solely responsible for the biological effects of these materials. The lack of information about these materials acts as the main limitation of this study. Thus, the components of these desensitizers should be further evaluated to better understand the basic mechanism/s of human gingival cell biological responses.

CONCLUSIONS

Within the limitations of this study, the results obtained indicate that hGFs show better *in vitro* biocompatibility after exposure to Clinpro White Varnish, even at the highest concentration employed, making it more eligible for topical applications. In contrast, Embrace Varnish exhibited a high cytotoxicity towards hGFs that could potentially delay the healing process and regeneration of the oral mucosa; more studies are needed to confirm this hypothesis.

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Regulatory Statement

The study protocol was approved by the Ethics Committee (Institutional Review Board for Human Subjects Research) of the University of Murcia (protocol ID: 2199/2018).

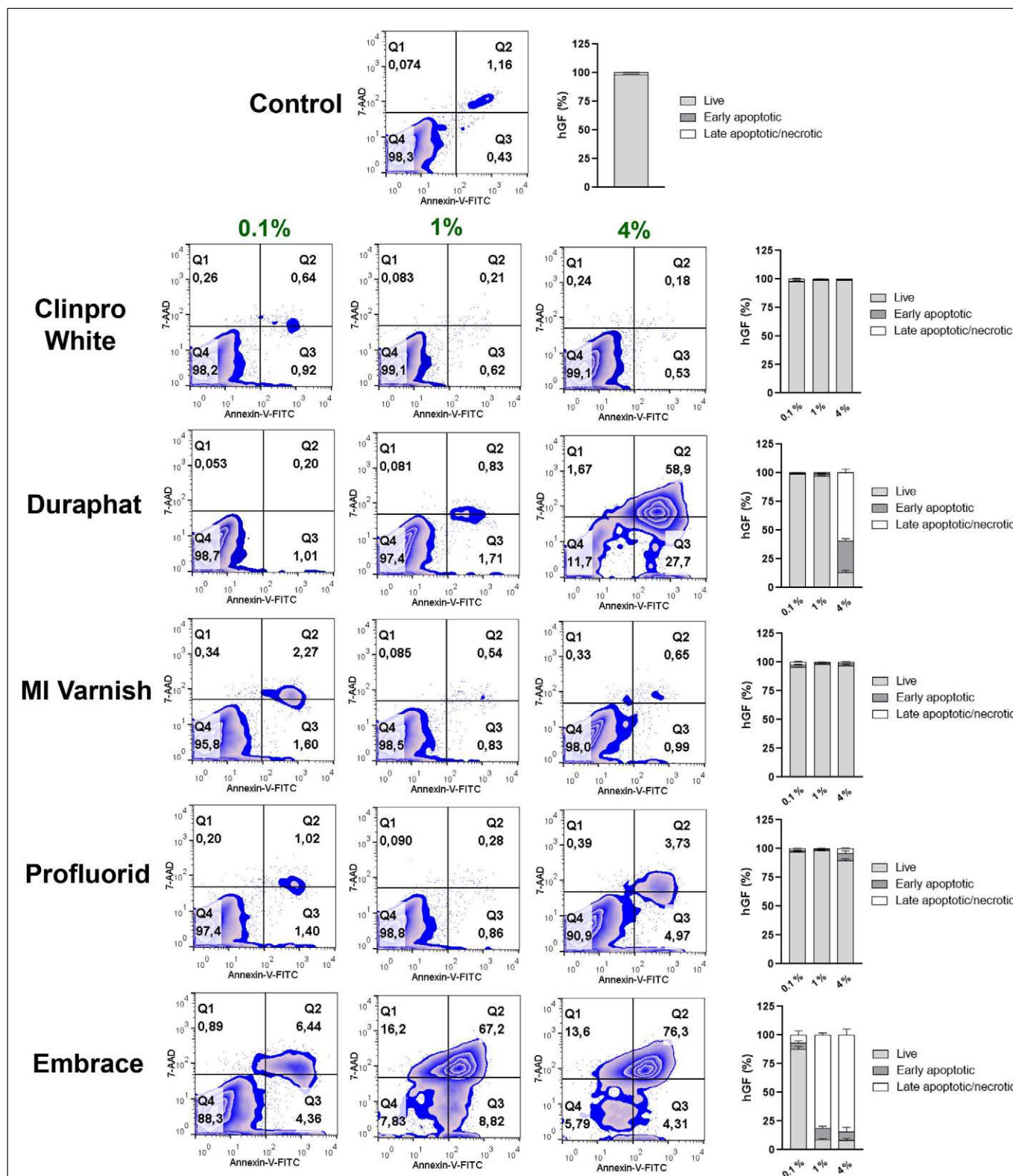


Figure 6. Apoptosis/necrosis assays. Human gingival fibroblasts (hGFs) were cultured in MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish eluates at different concentrations (0.1%, 1%, and 4%) for 72 hours at 37°C. Numbers within the different quadrants represent the percentages of live (Q4; Annexin-V-/7-AAD-), early apoptotic (Q3; Annexin-V+/7-AAD-), or late apoptotic and necrotic cells (Q2 and Q1; Annexin-V+/7-AAD+ and Annexin-V-/7-AAD+, respectively). Dot plots show representative flow cytometry results obtained from three independent experiments, and graphs show mean $\pm$ SD from $n = 3$ separate experiments.

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

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Challenging the Concept that OptiBond FL and Clearfil SE Bond in NCCLs Are Gold Standard Adhesives: A Systematic Review and Meta-analysis

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Clinical Relevance

The concept of gold standard dental materials should be re-evaluated. The evidence in the literature does not support the stating that OptiBond FL and Clearfil SE Bond are better than other materials in NCCLs.

SUMMARY

Purpose: The following PICO (Patient/Population, Intervention, Comparison, and Outcomes) question was proposed: “Are retention rates of composite resin restorations in noncarious cervical lesions (NCCLs) when using adhesives considered “gold

standard” (OptiBond FL and Clearfil SE Bond) higher than those obtained with other adhesives brands”? **Methods:** A search was performed in February 2019 (updated in November 2019) in the PubMed/MEDLINE, EMBASE, LILACS, BBO, Web of Science, Cochrane Library, Grey Literature, and IADR abstracts (1990-2018); unpublished and

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ongoing trial registries, dissertations, and theses were also searched. Only randomized clinical trials (RCTs) conducted in NCCLs that compared either OptiBond FL or Clearfil SE Bond adhesive with other commercially available adhesives were included. The risk of bias (RoB) was applied by using the Cochrane Collaboration tool. A meta-analysis was performed for retention rates at different follow-up times using a random effects model for both the adhesives. Heterogeneity was assessed with the Cochran Q test and I^2 statistics. Grading of Recommendations: Assessment, Development and Evaluation (GRADE) assessed the quality of evidence. Results: After removal of duplicates and noneligible articles, 25 studies remained for qualitative synthesis, as one study was common to the two adhesives, of which 9 studies were used for the OptiBond FL meta-analysis and 14 for the Clearfil SE Bond meta-analysis. No significant differences were observed for retention rates in follow-up periods of 12-24 months ($p=0.97$), 36-48 months ($p=0.72$), or 108-156 months ($p=0.73$) for OptiBond FL; and for 12-24 months ($p=0.10$) and 36-48 months ($p=0.17$) for Clearfil SE Bond. A significant difference was only found for OptiBond FL at 60-96 months ($p=0.02$), but only three studies were included in this meta-analysis. Conclusions: The evidence from available RCTs conducted in NCCLs that compared OptiBond FL or Clearfil SE Bond does not support the widespread concept that these adhesives are better than any other competitive brands available in the dental market.

INTRODUCTION

Two different bonding strategies can be used in adhesive procedures: the etch-and-rinse technique (ER) and the self-etch (SE) approach. ER adhesives require the previous demineralization of the dental substrates with a 32%-40% phosphoric acid etchant, followed by a primer and a bonding resin. If the primer and the bonding resin are separate steps, the adhesive is called a three-step ER system. If the priming and bonding are combined, the adhesive is called a two-step ER system.

The SE approach does not require a separate conditioning step, as the adhesive is theoretically capable of demineralizing and infiltrating the dental substrates simultaneously.¹ In the two-step SE approach, an acidic primer is applied before the application of a bonding resin, whereas, in the 1-step SE approach, the contents of the acidic primer and bonding resin are combined in a single-application solution.

From a clinical perspective, it is important to know which type of adhesive can provide the best performance. Systematic reviews have been published attempting to categorize the efficacy of the adhesive systems based on their bonding strategy and number of steps in noncarious cervical lesions (NCCLs).²⁻⁷ However, all material brands for each bonding strategy were grouped together, ignoring the fact that the efficacy of any adhesive depends on the material's composition, with some of them performing better than others in each bonding strategy.

Among the three-step ER systems, the adhesive named OptiBond FL (Kerr; Orange, USA), has been considered the gold standard material by many researchers⁸⁻¹¹ because of its good performance in immediate and long-term bond strength tests.¹² Some authors claim that the presence of glycerol phosphate dimethacrylate,¹³ which can interact chemically with the hydroxyapatite and the highly filled bonding resin layer (48 wt%) over the primed dental surfaces are responsible for this good performance.

As for as the SE strategy, the adhesive Clearfil SE Bond (Kuraray, Tokyo, Japan) is the one considered to be the gold standard material.^{10,14} This recognition was achieved because of the high bond strength values obtained in immediate and aged bonded interfaces.^{7,15,16} The presence of the 10-MDP monomer, which produces a strong and stable chemical bond, and which is less susceptible to degradation, is held to be the main factor in the good performance of this adhesive brand.¹⁷⁻¹⁹

If these materials are the gold standard, they should be better than other adhesive brands. Therefore, the aim of this systematic review and meta-analysis was to evaluate whether evidence from randomized clinical trials (RCTs) supports the designation of these products as gold standard materials. The following focused research question was posed based on the PICO acronym (P - participant, I - intervention, C - comparator, and O - outcome): "Are the retention rates of composite resin restorations in NCCLs better when "gold standard" (OptiBond FL or Clearfil SE Bond) adhesives are used compared with other brands?"

METHODS

Protocol and Registration

This study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO acknowledgement of receipt: 158813), and followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement.^{20,21}

Information Sources and Search Strategy

A search strategy for MEDLINE via PubMed based on the concepts of participant and intervention of the focused PICO question was elaborated. The strategy was adapted to other electronic databases (EMBASE, Cochrane Library, LILACS) and citation databases (Scopus and Web of Science) (Table 1). We did not restrict studies based on publication date and/or language.

Additionally, grey literature was investigated by searching the abstracts of the annual conference of the International Association for Dental Research (IADR) and its regional divisions (1990-2018), the database System for Information on Grey Literature in Europe and dissertations and theses using the ProQuest Dissertations and Theses full-text database as well as the Periódicos Capes Theses database.

Ongoing studies were searched in the following clinical trial registries: Current Controlled Trials (www.controlled-trials.com), International Clinical Trials Registry Platform (<http://apps.who.int/trialsearch/>), Clinical Trials Register (<http://www.clinicaltrials.gov>), ReBEC (www.rebec.gov.br), and European Union Clinical Trials Register (<https://www.clinicaltrialsregister.eu>). Additionally, we hand searched the reference lists of all primary and eligible studies of this systematic review for additional relevant publications. The first two pages of the related articles link of each primary study in the PubMed database were also examined.

Eligibility Criteria

We included only RCTs that compared the retention rates of the two so-called gold standard adhesives OptiBond FL or Clearfil SE Bond with other adhesive brands in NCCLs. We focused only on RCT in NCCLs, because this type of lesion was the one recommended by the American Dental Association²² for the evaluation of the clinical performance of adhesives. Studies were excluded if 1) adhesives did not follow the manufacturer's specifications, and 2) a comparative adhesive was not included.

Study Selection and Data Collection Process

The articles retrieved by the literature search were evaluated in three phases. All studies were initially scanned for relevance by title, and the abstracts of those that were not excluded at this stage were appraised. The next step included the abstract reading, and those articles not excluded had their full text retrieved for further evaluation. The full texts were then read by two reviewers to definitively check whether they met the inclusion criteria. Finally, the eligible articles received

a study identification (ID) by combining first author and year of publication.

The data were extracted using a standardized form in Excel 2016 (Microsoft; Redmond, WA, USA). Two reviewers independently abstracted information about the intervention from the included articles, including study design, participants, adhesives and composite resin types, mode of isolation of the operative field, cavity preparation (enamel beveling and dentin roughening), and number of examiners/operators and evaluation criteria. In case of disagreement, a decision was reached by consulting a third reviewer. Another worksheet containing the study identification and the outcomes per adhesive group at different follow-up periods was also prepared. If there were multiple reports of the same study (ie, reports with different follow-up times), data from all reports were extracted directly into a single data-collection form to avoid overlapping data.

Risk of Bias (RoB) in Individual Studies

The RoB of the eligible studies was evaluated by two independent reviewers by using the Cochrane Collaboration tool for assessing the RoB in RCTs (RoB version 1.0).²³ Disagreements were resolved by discussion.

We evaluated whether the randomization sequence and allocation concealment were adequate. Additionally, we evaluated whether blinding was implemented for participants, personnel, and outcomes assessment. Evidence of incomplete outcome data and selective reporting of outcomes was also checked. At the study level, they were judged to be at low RoB, if all domains were judged to be at low RoB.

Summary Measures and Synthesis of the Results

The main outcome evaluated was retention rate, and the meta-analysis was performed using the Meta package of the software R Analyses were carried out by using the random-effect model, and pooled-effect estimates were obtained by comparing the retention ratios of OptiBond FL and Clearfil SE Bond with those from other commercial brands of adhesives. We estimated the overall risk ratio (RR) for this binary outcome, presenting their 95% confidence intervals (CI) at different follow-up periods for each adhesive. Data from OptiBond FL and Clearfil SE Bond were summarized into the following follow-ups: 12-24 months and 36-48 months. For OptiBond FL, additional follow-ups (60-96 months and 108-156 months) were included, as these data were available. In case a study reported data twice within the range described above, data from the longest follow-up period were used.

Table 1: Database Search Strategy			
Database, Number of Papers: (5035)	Search (November 20, 2019)		
PubMed (1588)	dental restoration, permanent[MeSH Terms] OR dentition, permanent[MeSH Terms] OR tooth erosion[MeSH Terms] OR tooth erosion*[Title/Abstract] OR tooth abrasion[MeSH Terms] OR tooth abrasion*[Title/Abstract] OR dental abrasion*[Title/Abstract] OR tooth cervix[MeSH Terms] OR tooth cervix[Title/Abstract] OR abfraction*[Title/Abstract] OR cervical lesion*[Title/Abstract] OR NCCL*[Title/Abstract] OR class V[Title/Abstract] OR class 5[Title/Abstract] AND dentin-bonding agents[Mesh Term] OR adhesive system*[Title/Abstract] OR bonding agent*[Title/Abstract] OR dental adhesive*[Title/Abstract] OR adhesive material*[Title/Abstract] OR “etch-and-rinse adhesive”[Title/Abstract] OR “etch-and-rinse adhesives”[Title/Abstract] OR “total-etch adhesive”[Title/Abstract] OR “total-etch adhesives”[Title/Abstract] OR “self-etch adhesive”[Title/Abstract] OR “self-etching adhesive”[Title/Abstract] OR “self-etch adhesives”[Title/Abstract] OR “self-etching adhesives”[Title/Abstract] OR “all-in-one adhesive”[Title/Abstract] OR “all-in-one adhesives”[Title/Abstract] OR “one-bottle adhesive”[Title/Abstract] OR “one-bottle adhesives”[Title/Abstract] OR “single-bottle adhesive”[Title/Abstract] OR “single-bottle adhesives”[Title/Abstract] OR universal adhesive*[Title/Abstract] OR “multi-mode adhesive”[Title/Abstract] AND randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR “clinical trial”[tw] OR singl*[tw] OR doubl*[tw] OR trebl*[tw] AND mask*[tw] OR blind*[tw] OR placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR comparative study[pt] OR evaluation studies as topic		
Scopus (1626)	“t??th erosion” OR “t??th abrasion” OR “dental abrasion” OR “t??th cervix” OR “abfraction*” OR “cervical lesion*” OR “NCCL*” OR “class V” OR “class 5” AND “dentin bonding agent” OR “adhesive system” OR “bonding agent” OR “dental adhesive” OR “adhesive material” OR “etch-and-rinse adhesive” OR “self-etch adhesive” OR “self-etching adhesive” OR “all-in-one adhesive” OR “one-bottle adhesive” OR “single-bottle adhesive” OR “universal adhesive” OR “multi-mode adhesive”		
Cochrane Library (543)	#1	MeSH descriptor: [Dental Restoration, Permanent] explode all trees	1288
	#2	MeSH descriptor: [Dentition, Permanent] explode all trees	65
	#3	MeSH descriptor: [Tooth Erosion] explode all trees	222
	#4	(tooth next erosion):ti,ab,kw (Word variations have been searched)	233
	#5	MeSH descriptor: [Tooth Abrasion] explode all trees	124
	#6	tooth next abrasion (Word variations have been searched)	132
	#7	dental next abrasion (Word variations have been searched)	1
	#8	MeSH descriptor: [Tooth Cervix] explode all trees	292
	#9	tooth next cervix (Word variations have been searched)	306
	#10	abfraction (Word variations have been searched)	18
	#11	cervical next lesion (Word variations have been searched)	394
	#12	NCCL? (Word variations have been searched)	84
	#13	class next V (Word variations have been searched)	344
	#14	class next 5 (Word variations have been searched)	47
	#15	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14	1995
	#16	MeSH descriptor: [Dentin-Bonding Agents] explode all trees	937
	#17	adhesive next system (Word variations have been searched)	505
	#18	bonding next agent (Word variations have been searched)	1061
	#19	dental next adhesive (Word variations have been searched)	297

Table 1: Database Search Strategy (cont.)

Cochrane Library (543)	#20 adhesive next material (Word variations have been searched) 160 #21 "etch-and-rinse adhesive" (Word variations have been searched) 117 #22 "total-etch adhesive" (Word variations have been searched) 53 #23 "self-etch adhesive" (Word variations have been searched) 356 #24 "all-in-one adhesive" (Word variations have been searched) 25 #25 "one-bottle adhesive" (Word variations have been searched) 36 #26 "single-bottle adhesive" (Word variations have been searched) 13 #27 "universal next adhesive" (Word variations have been searched) 38 #28 "multi-mode adhesive" (Word variations have been searched) 3 #29 #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 1314 #30 #15 AND #29 Total: 545 (-2 reviews) = 543
Web of Science (688)	TS= ("t*th erosion" OR "t*th abrasion" OR "dental abrasion*" OR "tooth cervix" OR "abfraction*" OR "cervical lesion*" OR "NCCL" OR "class V" OR "class 5") AND TS= ("dentin bonding agent*") OR TS= ("adhesive system*") OR TS= ("bonding agent*") OR TS= ("dental adhesive*") OR TS= ("adhesive material*") OR TS= ("etch-and-rinse adhesive") OR TS= ("etch-and-rinse adhesives") OR TS= ("total-etch adhesive") OR TS= ("total-etch adhesives") OR TS= ("self-etch adhesive") OR TS= ("self-etch adhesives") OR TS= ("self-etching adhesive") OR TS= ("self-etching adhesives") OR TS= ("all-in-one adhesive") OR TS= ("all-in-one adhesives") OR TS= ("one-bottle adhesive") OR TS= ("one-bottle adhesives") OR TS= ("single-bottle adhesive") OR TS= ("single-bottle adhesives") OR TS= ("universal adhesive") OR TS= ("universal adhesives") OR TS= ("multi-mode adhesive")
LILACS (553)	mh:"dental restoration, permanent" OR "restauração dentária permanente" OR "restauración dental permanente" OR mh:"dentition, permanent" OR tw:"dentição permanente" OR "dentición permanente" OR mh:"tooth erosion" OR tw:"erosão dentária" OR tw:"erosión de los dientes" OR mh:"tooth abrasion" OR tw:"abrasão dentária" OR tw:"abrasión de los dientes" OR mh:"tooth cervix" OR tw:"colo do dente" OR tw:"cuello del diente" OR tw:abrac* OR tw:"cervical lesions" OR tw:"lesões cervicais" OR tw:"lesiones cervicales" OR tw:nccls OR tw:lcncs OR tw:"class V" OR tw:"class 5" OR tw:"classe V" OR tw:"classe 5" AND mh:"dentin bonding agents" OR tw:"adesivos dentinários" OR tw:"recubrimientos dentinarios" OR tw:"adhesive systems" OR tw:"sistemas adesivos" OR tw:"sistemas adhesivos" OR tw:"bonding agents" OR tw:"agentes de união" OR tw:"agentes de unión" OR tw:"dental adhesives" OR tw:"adesivos dentais" OR tw:"adhesivos dentales" OR tw:"adhesives materials" OR tw:"materiais adesivos" OR tw:"materiales adhesivos" OR tw:"etch and rinse adhesives" OR tw:"adesivos convencionais" OR tw:"adhesivos convencionales" OR tw:"total etch adhesives" OR tw:"condicionamento ácido total" OR tw:"adhesivos de grabado total" OR tw:"self etch adhesives" OR tw:"adesivos autocondicionantes" OR tw:"adhesivos autocondicionantes" OR tw:"self etching adhesives" OR tw:"all in one adhesives" OR tw:"adesivos de passo único" OR tw:"one bottle adhesives" OR tw:"adesivos de frasco único" OR tw:"single bottle adhesives" OR tw:"universal adhesives" OR tw:"adesivos universais" OR tw:"multi mode adhesives"
Embase (37)	"tooth erosion" OR "tooth abrasion" OR "dental abrasion" OR "tooth cervix" OR abfraction OR "cervical lesion" OR NCCL OR "class V" OR "class 5" AND "dentin bonding agent" OR "adhesive system" OR "bonding agent" OR "dental adhesive" OR "adhesive material" OR "etch-and-rinse adhesive" OR "self-etch adhesive" OR "self-etching adhesive" OR "all-in-one adhesive" OR "one-bottle adhesive" OR "single-bottle adhesive" OR "universal adhesive" OR "multi-mode adhesive"

Data were extracted using intention-to-treat analysis by using the total number of failures for each treatment arm in each follow-up as the nominator and the total number of participants randomized at baseline as the denominator, wherever trial reporting allowed. When trials had more than one adhesive brand being compared with the gold standards, they were included in the meta-analysis separately to provide more than one effect size; however, as in these situations, data from the control group were used more than once, the number of events and the total number of participants were divided among the comparisons to avoid a misleading overpowering of the estimates.²⁴

Trials have used several outcome measures; in those applying Vanherle, US Public Health Service or FDI World Dental Federation criteria, we dichotomized Alfa vs. Bravo/Charlie, and these last two were counted as failures.

Only studies classified at low or unclear RoB were meta-analyzed. Heterogeneity was evaluated using the Cochran Q test and I^2 statistics. The 95% prediction interval was calculated in all meta-analyses with at least five studies. Sensitivity analyses were also conducted to investigate the reasons for high heterogeneity whenever detected.

Assessment of the Quality of Evidence Using GRADE

The quality of the evidence was graded for each outcome across studies (body of evidence) using the Grading of Recommendations: Assessment, Development and Evaluation (GRADE) (<http://www.gradeworkinggroup.org/>). This technique allowed determination of the overall strength of evidence for each meta-analysis.²⁵ The GRADE grades the evidence into four levels: very low, low, moderate, and high. The “high-quality” level suggests high confidence that the true effect lies close to the estimate of the effect. At the other extreme, “very-low-quality” level suggests very low confidence in the effect estimate, and the estimate reported can be substantially different from what was measured.

For RCTs, the GRADE approach addresses five reasons (RoB, imprecision, inconsistency, indirectness of evidence, and publication bias) for possibly rating down the quality of the evidence by one or two levels. Each of these aspects was assessed as having “no limitation” (0); “serious limitations” (1 level downgraded), and “very-serious limitations” (2 levels downgraded). The GRADEpro Guideline Development Tool, available online (www.grade-pro.org), was used to create a summary-of-findings table as suggested in the Cochrane Handbook for Systematic Reviews of Interventions.

RESULTS

Study Selection

The search strategy was conducted initially on February 9, 2019 and was updated on November 20, 2019. A total of 5058 publications were retrieved in all databases. After database screening and duplicate removal, 689 studies were identified, 155 of which were retrieved for further assessment, because they appeared to be relevant. A flowchart outlining the study selection process according to the PRISMA Statement²⁶ can be seen in Figure 1. Of these, 122 were not included for various reasons (Supplementary Table 1), leaving 33 eligible RCTs. From these 33 articles, 8 studies^{9,27-33} reported the same study sample at different follow-ups and, therefore, received the same study ID. Therefore, 25 studies were eligible for inclusion, with one study being common for both adhesives.³⁴

Characteristics of the Included Studies

The characteristics of the 25 eligible studies are listed in Table 2. Most studies performed multiple restorations per participant, and only one did not report this information.³⁵ OptiBond FL was compared with eight different commercial brands of adhesives in the eligible studies as follows:

- Clearfil S3 Bond (Kuraray)³⁶
- Clearfil SE Bond (Kuraray)³⁴
- G-Bond (GC; Tokyo, Japan)^{37,38}
- OptiBond All-in-One (Kerr)^{39,40}
- OptiBond Solo Plus (Kerr)^{39,40}
- OptiBond XTR (Kerr)^{39,41}
- PermaQuick (Ultradent; UT, USA)⁴²
- Scotchbond Universal (3M Oral Care; MN, USA)⁴³

The adhesive Clearfil SE Bond was compared with 14 different commercial brands as described below:

- AdheSE (Ivoclar Vivadent; Schaan, Liechtenstein)⁴⁴
- Admira Bond (Voco; Cuxhaven, Germany)⁴⁵
- Adper Prompt (3M Oral Care)⁴⁶
- Clearfil S3 Bond (Kuraray)⁴⁷
- G-Bond (GC)⁴⁸
- Hybrid Bond (Sun Medical; Shiga, Japan)⁴⁵
- OptiBond FL (Kerr)³⁴
- PQ1 (Ultradent)⁴⁹
- Prime & Bond NT (Dentsply Sirona; Konstanz, Germany)⁵⁰
- Scotchbond Universal (3M Oral Care)⁵¹
- Single Bond (3M Oral Care)⁵¹⁻⁵³
- Xeno III (Dentsply Sirona)⁵³

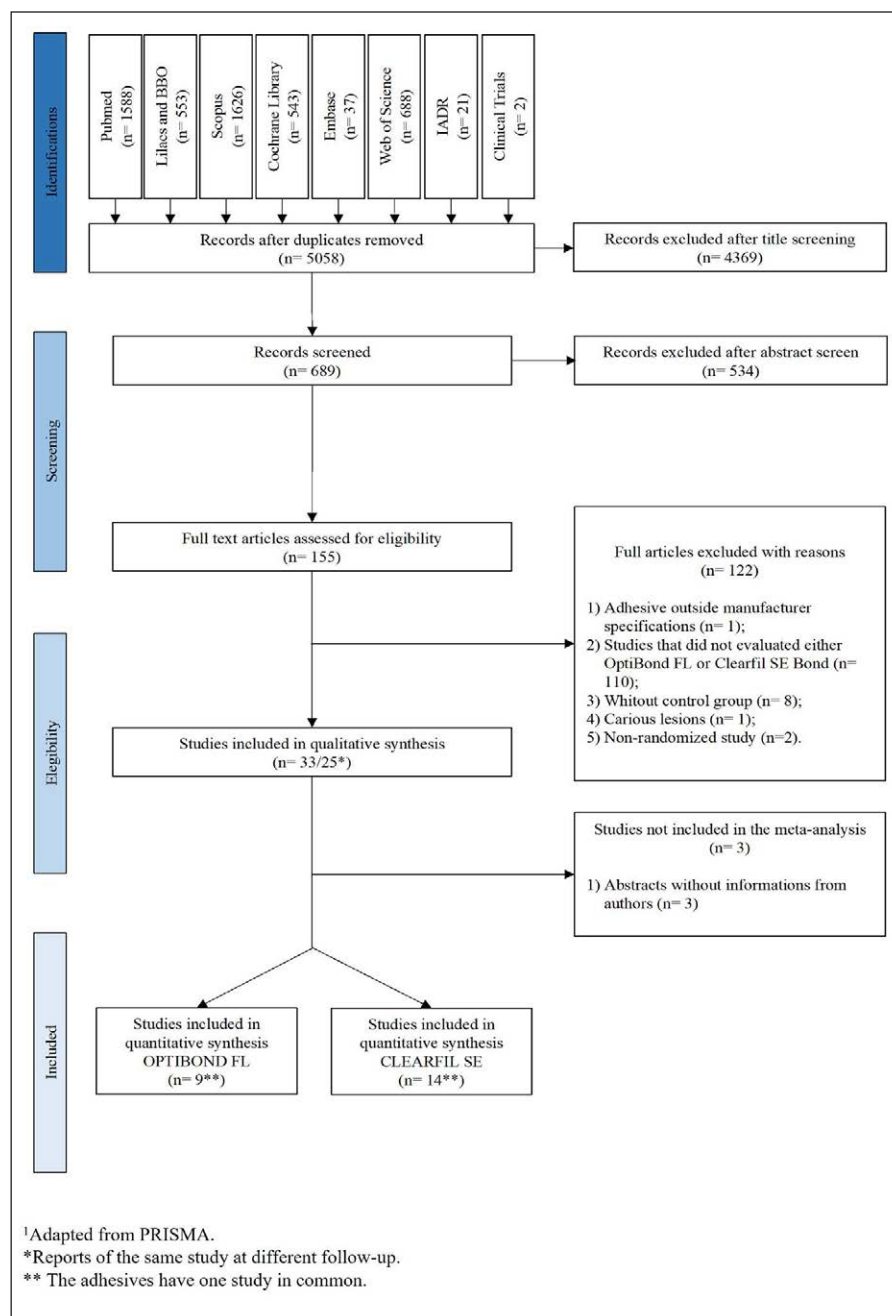


Figure 1. Flow diagram of study identification (*Reports of the same study at different follow-up; ** The adhesives have one study in common.)

- Xeno V (Dentsply Sirona)^{54,55}
- XP-Bond (Dentsply Sirona)⁵⁶

Most of the composite resins used were microhybrids, nanohybrids, or nanofilled composite resins. The following commercial brands were used: Premise (Kerr),⁵⁷ Estelite Sigma Quick (Tokuyama; Tokyo, Japan),⁵⁷ CeramX-Duo (Dentsply Sirona),⁵⁸ Filtek Z-250 (3M Oral Care),^{35,44,54} Filtek Supreme Ultra (3M Oral Care),³⁹ Clearfil AP-X (Kuraray),^{36,45-48,50} Filtek Supreme

XTE (3M Oral Care),⁴³ Herculite XRV (Kerr),⁴⁰ Grandio (Voco),³⁴ Herculite Ultra (Kerr),⁴¹ Venus Diamond (Kulzer; Hanau, Germany),³⁷ Gradia Direct (GC),³⁸ Amelogen Hybrid (Ultradent),⁴² Amelogen Microfill (Ultradent),⁴² Prodigy (Kerr),⁴² Filtek A-110 (3M Oral Care),⁵² Clearfil ST (Kuraray),⁵² Filtek Supreme (3M Oral Care),^{51,53} Esthet-X (Dentsply Sirona),⁵⁵ Filtek Z-350 (3M Oral Care),⁴⁶ TPH Spectrum (Dentsply/De Trey),⁵⁰ Tetric Ceram (Ivoclar Vivadent),⁴⁹ Point 4 (Kerr),⁴⁹ and Ceram X Mono (Dentsply Sirona).⁵⁶

Table 2: Summary of the Descriptive Characteristics of the Primary Studies Included for Each Adhesive System (n=26/25 ^a)			
Study ID	Study Design [setting]	Follow-ups (mth)	Subject's Age Mean $\pm$ SD [range] (yrs)
OPTIBOND FL			
Armstrong 2012 ⁵⁷	Paired [university]	6, 12	n.r. $\pm$ n.r. [n.r.]
Blunck 2013 ⁵⁸	Paired [n.r.]	12, 24	n.r. $\pm$ n.r. [n.r.]
Dall'orologio 2006, ³⁵ 2008, ²⁷ 2009, ²⁹ 2010 ²⁸	n.r. [n.r.]	Baseline, 6, 12, 18, 24, 36, 60, 78, 84	n.r. $\pm$ n.r. [n.r.]
de Paula 2015 ³⁹	Multiple restorations [university]	Baseline, 6, 12	n.r. $\pm$ n.r. [20->49]
Ermis 2012 ³⁶	Multiple restorations [university]	Baseline, 6, 12, 24	50 $\pm$ 8.3 [39-79]
Haak 2019 ⁴³	Paired [university]	Baseline, 6, 12	65 $\pm$ 20.5 [43-84]
Moosavi 2013 ⁴⁰	Paired [university]	Baseline, 6, 12, 18	n.r. $\pm$ n.r. [20-50]
Mortazavi 2012 ^{34a}	Paired [university]	Baseline, 6, 9, 12	n.r. $\pm$ n.r. [30-60]
Ritter 2015 ⁴¹	Multiple restorations [university]	Baseline, 6, 18	n.r. $\pm$ n.r. [n.r.]
Scotti 2016 ³⁷	Multiple restorations [university]	12, 24, 36	52.4 $\pm$ n.r. [32-63]
Van Landuyt 2008, ³⁸ 2011, ⁹ 2014, ³⁰ Peumans 2018 ³¹	Multiple restorations [university]	Baseline, 6, 12, 24, 36, 60, 108	n.r. $\pm$ n.r. [20->80]
Van Meerbeek 2004, ⁴² Peumans 2007, ³² 2012 ³³	Paired [university]	6, 12, 24, 36, 60, 84, 156	n.r. $\pm$ n.r. [<18->70]
Abbreviations: #, number; FDI, World Dental Federation; ID, identification; mth, month; n.r., not reported; SD, standard deviation; USPHS, United State Public Health Service.			
^a One study common to the two adhesives.			
^b Depending on access and location of the lesion.			

Table 2: Summary of the Descriptive Characteristics of the Primary Studies Included for Each Adhesive System (n=26/25^a) (cont.)

Groups: Type of Adhesive -Adhesive Brand [Number of Restorations per Group]	Resin Composite per Group	Rubber Dam	Enamel Bevel/ Dentin Prep	# of Operators/ Examinators	Evaluation Criteria
3ER- Optibond FL [30] 1SE- Tokuyama Bond Force [30]	Premise Estelite Sigma Quick	No/Yes ^b	n.r./n.r.	n.r./n/r	USPHS
1SE- iBond [58] 1SE- G-Bond [58] 1SE- Clearfil S3 Bond [58] 3ER- Optibond FL [58]	CeramX-Duo ⁱ	n.r.	No/No	2/n.r.	USPHS
1SE- iBond [n.r.] 1SE- AQ Bond [n.r.] 3ER- Optibond FL [n.r.]	Filtek Z-250	n.r.	n.r./n.r.	3/1	Modified USPHS
3ER- Optibond FL [46] 2ER- Optibond Solo Plus [44] 2SE- Optibond XTR [44] 1SE- Optibond All-in-One [46]	Filtek Supreme Ultra	Yes	No/No	04/02	FDI/ Modified USPHS
1SE- Clearfil S3 Bond [81] 3ER- Optibond FL [80]	Clearfil AP-X	No	Yes/Yes	01/02	Vanherle
1SE- Scotchbond Universal [110] 3ER- Optibond FL [55]	Filtek Supreme XTE	No	Yes/Yes	01/01	FDI
3ER- Optibond FL [30] 2ER- Optibond Solo Plus [30] 1SE- Optibond All-in-One [30]	Herculite XRV	No	No/No	01/02	USPHS
2SE- Clearfil SE Bond [12] 3ER- Optibond FL [12]	Grandio	Yes	Yes/No	n.r./02	USPHS
2SE- Optibond XTR [41] 3ER- Optibond FL [42]	Herculite Ultra	Yes/No ^b	No/Yes	4/2	Modified USPHS
1SE- G-Bond [46] 3ER- Optibond FL [44]	Venus Diamond	Yes	Yes/Yes	02/02	Modified USPHS
1SE- G-Bond [133] 3ER- Optibond FL [134]	Gradia Direct	No	Yes/Yes	02/02	Vanherle
3ER- PermaQuick [100] 3ER- Optibond FL [50]	Amelogen Hybrid Amelogen Microfill Prodigy	Yes	Yes/No	02/02	Vanherle

Table 2: Summary of the Descriptive Characteristics of the Primary Studies Included for Each Adhesive System (n=26/25^a) (cont.)

Study ID	Study Design [setting]	Follow-ups (mth)	Subject's Age Mean $\pm$ SD [range] (yrs)
CLEARFIL SE BOND			
Abdalla & Garcia-Godoy 2006 ⁴⁵	Paired [n.r.]	12, 24	n.r. $\pm$ n.r. [35-52]
Araújo 2015 ⁴⁴	Multiple restorations [university]	Baseline, 24	45 $\pm$ 8 [n.r.]
Brackett 2010 ⁴⁷	Paired [university]	Baseline, 6, 12, 24	n.r. $\pm$ n.r. [31-58]
Burrow & Tyas 2007 ⁵²	Multiple restorations [university]	6, 12, 24, 36	61 $\pm$ n.r. [n.r.]
Dalkilic e Omurlu 2012 ⁵³	Multiple restorations [university]	Baseline, 3, 12, 24	n.r. $\pm$ n.r. [30-70]
Jang 2017 ⁵⁴	Multiple restorations [university]	Baseline, 6, 12, 18, 24	55 $\pm$ n.r. [30-73]
Mortazavi 2012 ^{34b}	Paired [university]	Baseline, 6, 9, 12	n.r. $\pm$ n.r. [30-60]
Pena 2016 ⁵⁵	Paired [university]	Baseline, 3, 6, 12, 18, 24	n.r. $\pm$ n.r. [n.r.]
Qin 2013 ⁴⁶	Multiple restorations [university]	Baseline, 6, 12, 24	44.1 $\pm$ n.r. [27-66]
Türkün 2003 ⁵⁰	Paired [university]	Baseline, 6, 12, 24	46 $\pm$ n.r. [26-60]
van Dijken 2010 ⁴⁹	Multiple restorations [university]	Baseline, 6, 12, 18, 24, 36, 48, 60, 72, 84, 96	60.1 $\pm$ n.r. [42-84]
Yaman 2014 ⁵⁶	Paired [university]	Baseline, 6, 12, 24, 36	45.12 $\pm$ n.r. [32-58]
Zanatta 2019 ⁵¹	Paired [university]	Baseline, 6, 12, 24	n.r. $\pm$ n.r. [<20->60]
Zhou 2009 ⁴⁸	Multiple restorations [university]	Baseline, 3, 6, 12	n.r. $\pm$ n.r. [n.r.]

Table 2: Summary of the Descriptive Characteristics of the Primary Studies Included for Each Adhesive System (n=26/25^a) (cont.)

Groups: Type of Adhesive -Adhesive Brand [Number of Restorations per Group]	Resin Composite per Group	Rubber Dam	Enamel Bevel/ Dentin Prep	# of Operators/ Examinators	Evaluation Criteria
2ER- Admira Bond [65] 2SE- Clearfil SE Bond [65] 1SE- Hybrid Bond [65]	Clearfil AP-X	Yes	No/Yes	01/02	USPHS
2SE- Clearfil SE Bond [32] 2SE- AdheSE [32]	Filtek Z-250	No	No/No	01/02	Modified USPHS
2SE- Clearfil SE Bond [40] 1SE- Clearfil S3 Bond [40]	Clearfil AP-X	No	No/No	02/02	Modified Ryge/ USPHS
2ER- Single Bond [30] 2SE- Clearfil SE Bond [30]	Filtek A-110 Clearfil ST	n.r.	No/No	02/01	n.r.
2ER- Single Bond [60] 2SE- Clearfil SE Bond [71] 1SE- Xeno III [60]	Filtek Supreme	No	No/Yes	01/01	Modified USPHS
2SE- Clearfil SE Bond [83] 1SE- Xeno V [81]	Filtek Z-250	No	No/No	n.r./02	Modified FDI
2SE- Clearfil SE Bond [12] 3ER- Optibond FL [12]	Grandio	Yes	Yes/No	n.r./02	USPHS
2SE- Clearfil SE Bond [56] 1SE- Xeno V [56]	Esthet-X	No	Yes/No	01/02	Modified USPHS
2SE- Clearfil SE Bond [58] 1SE- Adper Prompt [56]	Clearfil AP-X Filtek Z-350	No	Yes/Yes	02/02	Modified USPHS
2SE- Clearfil SE Bond [49] 2ER- Prime & Bond NT [49]	Clearfil AP-X TPH Spectrum	No	No/Yes	01/02	USPHS
2SE- Clearfil SE Bond [55] 2ER- PQ1 [64]	Tetric Ceram Point 4	No	No/Yes	01/03	Modified USPHS
2SE- Clearfil SE Bond [48] 2ER- XP Bond [48]	Ceram X mono	No	Yes/Yes	02/02	Modified USPHS
2ER- Scotchbond Universal [38] 1SE- Scotchbond Universal [38] 2ER- Single Bond [38] 2SE- Clearfil SE Bond [38]	Filtek Supreme	No	No/No	04/02	FDI
1SE- Clearfil 3S Bond [124] 2SE- Clearfil SE Bond [124] 1SE- G-Bond [94]	Clearfil AP-X	Yes	Yes/Yes	n.r./02	Modified USPHS

The majority of the studies reported that no rubber dam had been applied,^a six studies reported the use of rubber dam,^{34,37,39,42,45,48} another three did not report this information,^{35,52,58} and two studies stated that rubber dam was used depending on the location and access to the lesion.^{41,57}

Ten studies^b prepared a small enamel bevel at the incisal/occlusal margin of the lesion, 11 studies^c superficially roughened the exposed dentin with a coarse diamond rotatory instrument, 8 studies^d did not prepare either the enamel or dentin, and 2 studies^{35,57} did not report this information.

Risk of Bias in the Included Studies

The quality assessment of the RoB of included studies both for OptiBond FL and Clearfil SE Bond is presented in Figures 2 and 3. From the 12 eligible studies that evaluated OptiBond FL, only 1³⁹ was considered at low RoB, while the other 11 were considered at unclear RoB. From the 14 studies that evaluated the Clearfil SE Bond studies, 2 studies^{44,51} were considered low RoB, and the remaining were at unclear risk.

Meta-analyses

OptiBond FL

Nine studies were included in the meta-analysis, as three studies were excluded because of lack of data (they were abstracts).^{35,57,58} One study provided three effect sizes, as OptiBond was compared with three different adhesives,³⁹ and another provided two effect sizes.⁴⁰ The results are presented in Figure 4. No significant differences in the retention rates between groups (OptiBond FL vs other adhesive brands) were observed at the follow-ups of 12-24 months, 36-48 months, and 108-156 months ($p>0.72$). The risk ratio (RR) and the 95% confidence interval at 12-24 months was 0.99 (0.56-1.75; $p=0.97$), the RR and the 95% confidence interval at 36-48 months was 1.12 (0.61-2.03; $p=0.72$), and the RR and the 95% confidence interval at 108-156 months was 0.94 (0.67-1.32; $p=0.73$), respectively. A significant difference was found between the groups at 60-96 months, with an average RR of 1.65 (1.07-2.53; $p=0.02$) in favor of the OptiBond FL. Heterogeneity was not observed in any of the follow-up periods ($I^2=0$; $p>0.50$).

Clearfil SE Bond

All the 14 studies were included in the meta-analysis, and the results are presented in Figure 5. Four

studies provided two effect sizes.^{45,48,51,53} No significant differences in the retention rates between groups (Clearfil SE Bond vs other adhesives) were observed in any of the different follow-up periods ($p>0.10$). The RR and the 95% confidence interval at 12-24 months was 1.21 (0.97-1.53; $p=0.10$) and the RR and the 95% confidence interval at 36-48 months was 1.49 (0.84-2.67; $p=0.17$), respectively. Heterogeneity was observed at the follow-up periods to 36-48 months ($p=0.09$; $I^2=59\%$).

Assessment of the Quality of Evidence (GRADE)

The quality of evidence assessed for both the OptiBond FL and Clearfil SE Bond in all study follow-ups was considered to be low due to limitations in the RoB of the eligible studies (most were at unclear risk) and due to imprecision (Table 3). Although the short-term meta-analysis included more than 10 comparisons, the number of events in the short-term follow-ups was quite low, leading to imprecision. In the medium- to long-term follow-ups, imprecision was mainly attributed to the low number of studies and the consequent wide confidence interval around the point estimate.

DISCUSSION

Well-done systematic reviews, with or without meta-analysis, are generally considered to provide the best evidence for the type of study design summarized, as they are based on the findings of multiple studies identified in comprehensive and systematic literature searches. As stated in the introduction section, pairwise meta-analysis involving adhesive systems in NCCLs commonly compare adhesive strategies^{4,5,7,59} rather than specific material brands. In these reviews, the so-called 'gold standard' adhesives were grouped with other adhesive brands within their classification group, and, therefore, their individual performance cannot be assessed. That is, variables such as solvents, initiator systems, functional monomers, structural monomers, hydrophilic monomers, number of steps, and mode of application of each adhesive present in the primary studies were not evaluated in the present review. Any attempt to associate one of these factors with the findings of this article could lead to spurious results of an observational nature and be subject to several confounding factors.

Other issues such as enamel beveling and dentin roughening could not be investigated in the present systematic reviews; however, an earlier systematic review showed that we do not have evidence to state that enamel beveling has any role on restoration retention in NCCL.⁶⁰ Similarly, a RCT comparing dentin roughening with not dentin roughening ended up with the conclusion that this procedure does not affect the retention rates of composite resins in NCCLs.⁶¹

^a 36,38,40, 43,44,46,47,49-51,53-56

^b 34,36-38,42,43,46,48,55,56

^c 36-38,41,43,45,46,48-50,53

^d 39,40,44,47,51,52,54,58

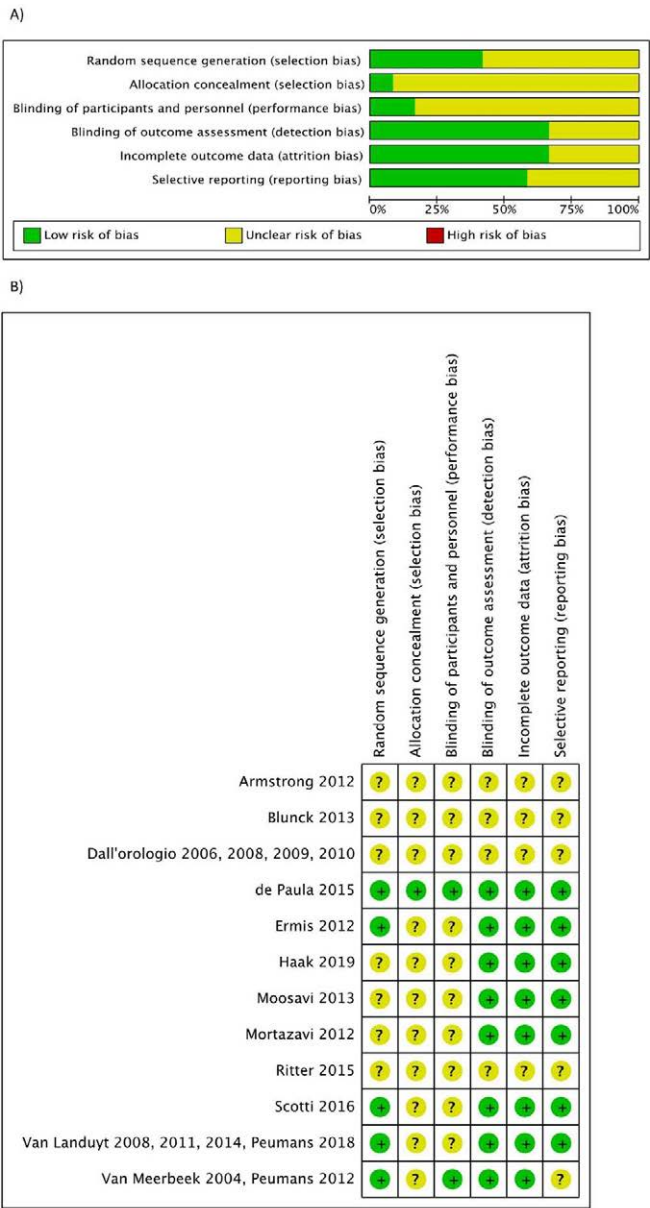


Figure 2. (A): Risk of bias graph for OptiBond FL according to the Cochrane Collaboration Tool. (B): Risk of bias summary for OptiBond FL.

The authors are unaware of a previous systematic review that compared retention rates of specific brands of adhesive systems with other competitive adhesive materials. Studies ranging from 2003 to 2019 were found and the results contained in them challenge the widespread concept that the three-step ER OptiBond FL, and the two-step SE Clearfil SE are “gold standard” materials. Their overall retention rates were not better than the overall retention rates of other competitive adhesive brands with which they were compared, except at 60-96 months for OptiBond FL. In this follow-

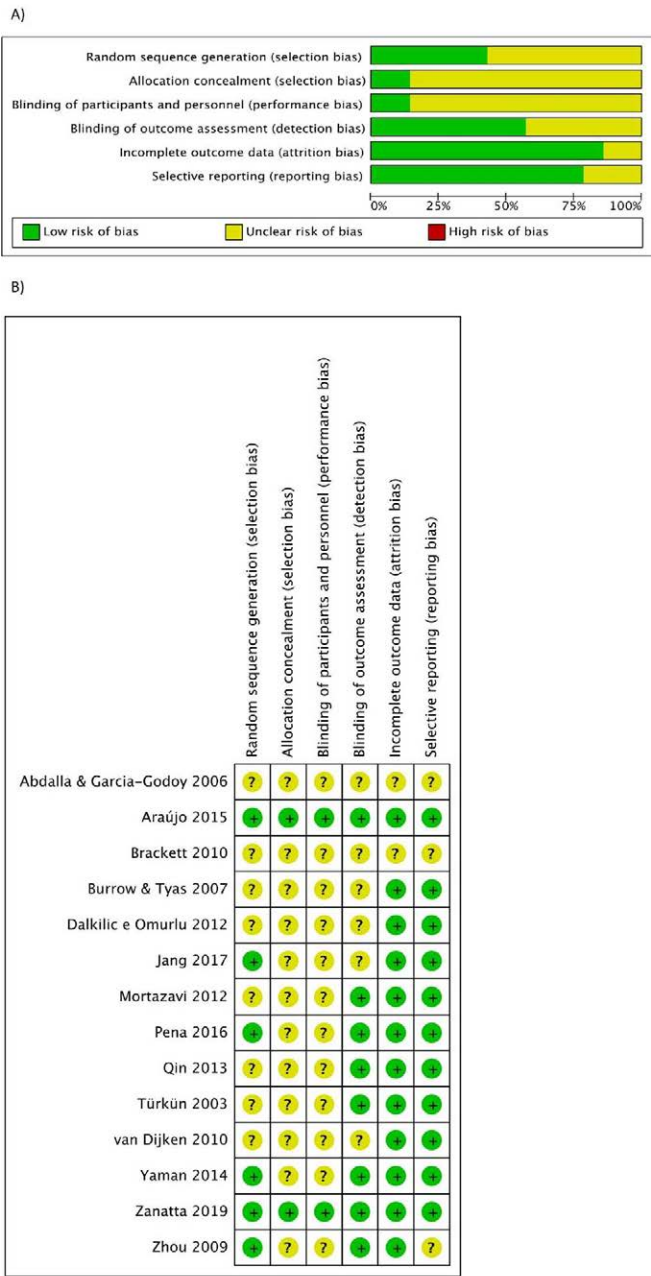


Figure 3. (A): Risk of bias graph for Clearfil SE Bond according to the Cochrane Collaboration Tool. (B): Risk of bias summary for Clearfil SE Bond.

up, only two studies were included, therefore providing an imprecise estimate.

Although this concept has been used for more than 20 years in the literature, it became a stronger evidence for the publication of a meta-analytical review of parameters on bond strength values.¹⁰ In their review of laboratory studies,¹⁰ the authors concluded that the best-performing adhesive both in short- and long-term studies was the three-step ER OptiBond FL, while the

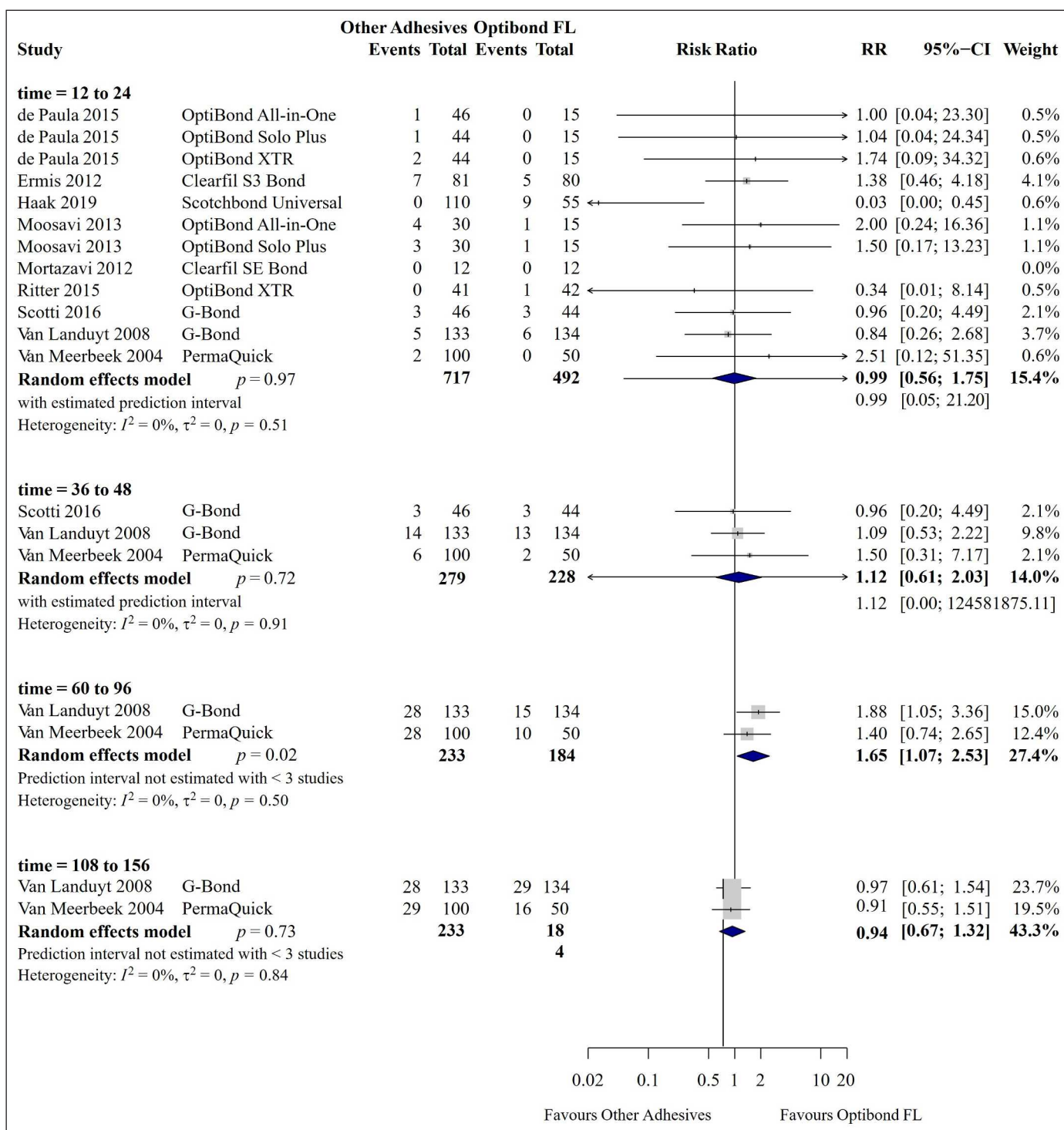


Figure 4. Forest plots of the retention rates for OptiBond FL at 12 to 24-month, 36 to 48-month, 60 to 96-month and 108 to 156-month.

second-best performance adhesive was the two-step SE Clearfil SE Bond.

The lack of agreement between the meta-analytical review¹⁰ and the present meta-analysis of RCTs suggests that laboratory and clinical findings are not consistent.

While an earlier study reported correlation between laboratory and clinical data, one cannot exclude the fact that this reported correlation was spurious, as it was only found between “aged” bond-strength data with medium-term retention rates of adhesives.¹⁴

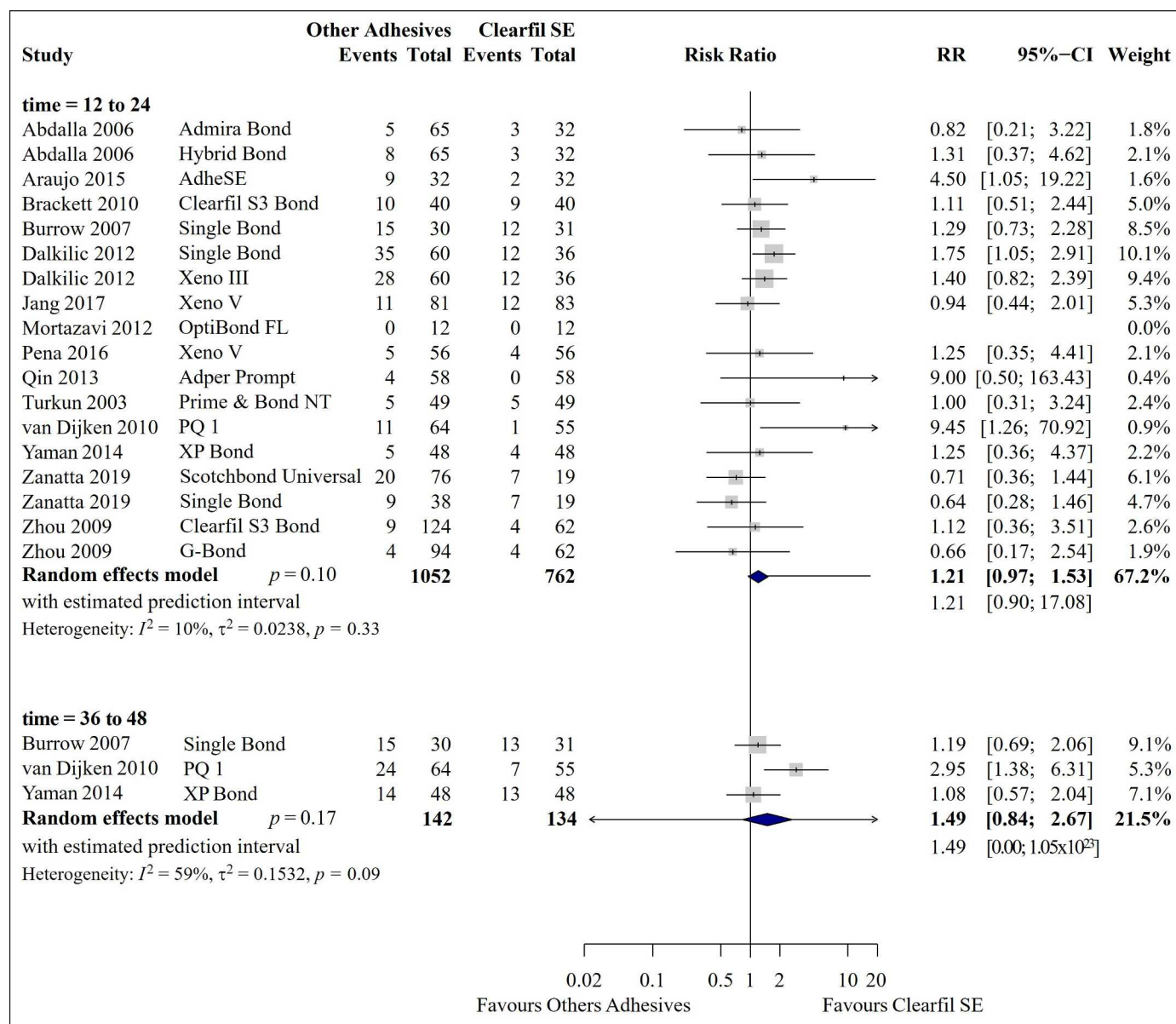


Figure 5. Forest plots of the retention rates for Clearfil SE Bond at 12 to 24-month and 36 to 48-month.

Nevertheless, this conclusion does not mean that the adhesives OptiBond FL and Clearfil SE Bond are not good adhesives. Indeed, they have good clinical performance with clinical data of up to 13 years of follow-up.^{62,63} Very few adhesive systems have been followed up for such a long time. However, the merged data of OptiBond FL and Clearfil SE Bond from eligible RCTs in short- to long-term studies have not shown superiority of these materials over other brands, suggesting that there are other adhesives as good as these two. We do agree with some authors^{17,64} that the clinical performance of the adhesive system is not related to the bonding strategy but to the product chemical

composition. Therefore, any attempt to categorize the efficacy of adhesives based on their classification may be misleading. This may explain why systematic reviews grouping brands of adhesive systems from the same category failed to reach a consensus.^{5,7,59}

Some considerations about the RCTs included in this systematic review are required. Authors of RCTs of bonding studies have not reported the study findings in a standardized way, and this may lead to misleading conclusions. In some clinical trials, events at the shortest follow-ups are not carried forward to the longest follow-ups, which may lead to the reporting of misleading results. This misleading report probably

Table 3: Summary of Findings Table for OptiBond FL and Clearfil SE Bond						
Retention Rates		Anticipated Absolute Effects ^a (95% CI)		Relative Effect (95% CI) ^a	Number of Participants (Studies)	Certainty of the Evidence (GRADE) ^b
		Risk with Other Competitive Brands	Risk with Optibond FL			
OptiBond FL	Follow up range 12-24 month	39 per 1000	39 per 1000 (22-68)	RR 0.99 (0.56-1.75)	1209 (12 RCTs)	□□□□ LOW a,b
	Follow up range 36-48 month	82 per 1000	92 per 1000 (50-167)	RR 1.12 (0.61-2.03)	507 (3 RCTs)	□□□□ LOW a,c
	Follow up range 60-96 month	240 per 1000	397 per 1000 (257-608)	RR 1.65 (1.07-2.53)	417 (2 RCTs)	□□□□ LOW a,d
	Follow up range 108-156 month	245 per 1000	230 per 1000 (164-323)	RR 0.94 (0.67-1.32)	417 (2 RCTs)	□□□□ LOW a,e
Clearfil SE Bond	Follow up range 12-24 month	183 per 1000	222 per 1000 (178-281)	RR 1.21 (0.97-1.53)	1814 (18 RCTs)	□□□□ LOW a,e
	Follow up range 36-48 month	373 per 1000	556 per 1000 (314-997)	RR 1.49 (0.84-2.67)	276 (3 RCTs)	□□□□ LOW a,e
Abbreviations: CI, confidence interval; RR, risk ratio. ^aThe risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). ^bGRADE Working Group grades of evidence High certainty: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect. Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.						

occurred because the recall rate drops drastically in long-term follow-ups. In these situations, the review authors calculated the retention rates based on the number of recalled restorations and not based on the total number of restorations placed at baseline.

When we collected data for this meta-analysis, we used the total number of restorations at baseline as a reference for all studies. This approach assumes that none of those missed at the follow-up suffered the target outcome, ie, debonding. Making this assumption, we presented the study results as the best estimate, as we do not know (like the authors of the eligible studies) what happened to the unseen restorations. Although this approach may overestimate the retention rates of

the adhesives, it does not break the random assignment to the treatment groups in the studies.

The other alternative would be to use the number of restorations recalled at each follow-up as a denominator of the retention rate; however, this number is not always provided. Some authors report an overall recall rate and not the recall rate per group,⁵⁵ which does not help in data extraction; others do not report the recall rate at all.^{34,40} To make the scenario harder, some study authors report the percentage of events instead of the raw numbers, without specifying whether the denominator of such percentages is the total number of placed restorations at baseline or the total number of evaluated restorations at any follow-up.

All these concerns regarding data extraction indicate an urgent need for standardization of the report of studies conducted on NCCLs. Instead of providing retention rates per follow-up, the use of survival analysis could provide better estimates of what occurs to the adhesives over time.

Another important issue to be addressed is that the great majority of the studies had an unclear RoB. To prevent selection bias, the randomization process should be adequately performed, and the random sequence should be protected from foreknowledge until the implementation of the intervention. The latter procedure is called allocation concealment. Only 30%-40% of eligible studies from this review reported the randomization process used in their study, and only 10%-15% of the studies adequately reported the allocation concealment.

The poor reporting of the random sequence generation and the allocation concealment in studies conducted in NCCLs has been highlighted in a previous review that assessed the compliance of bonding studies in NCCLs to the CONSORT statement,⁶⁵ which is a set of recommendations for reporting clinical trials in biomedical literature. This fact led us to downgrade the quality of evidence by one level for the RoB.

Another important consideration is that most of the RCTs focused on short- and medium-term follow-ups, ie, 12-48 months for both the adhesives. In these short-term follow-ups, the number of events, ie, debonded restorations, was low for both the groups, leading to imprecise estimates. The body of evidence produced at these follow-ups was also downgraded for imprecision due to the low number of events. Similarly, the long-term follow-ups were downgraded for imprecision, but due to the low number of studies and wide 95% confidence interval.

Only three medium- to long-term studies (5 years or more) for OptiBond FL were found. Clearfil SE Bond was not compared with other adhesive brands in follow-ups equal to or longer than 5 years. The studies of Van Meerbeek 2005⁶⁶ and Peumans 2005,⁸ 2007,⁶⁷ 2010,⁶⁸ 2015⁶³ evaluated the long-term performance of this two-step SE adhesive, but the authors did not compare it with other adhesive brands, which prevented a meta-analysis of these data.

Based on the available RCTs meta-analyzed in this systematic review, there are adhesives whose performance is similar to that of those currently considered "gold standard" adhesives in the dental market. However, the quality of the body of evidence was considered low, and further RCTs, mainly with a long-term record of performance, are required.

CONCLUSION

We have no evidence from the available RCTs that compared OptiBond FL or Clearfil SE Bond, used in NCCLs, to support the widespread concept that these adhesives are better than other competitive brands available in the dental market.

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Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

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Effects of Remineralizing Agents Based on Calcium Phosphate, Sodium Phosphate, or Sodium Fluoride on Eroded Cervical Dentin

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Clinical Relevance

Calcium phosphate-based remineralizing agents can prevent the harmful effects of erosion challenges on the dentin collagen matrix. In addition, they may be a promising and viable alternative for treatment of dentin hypersensitivity—and prevent the harmful impacts of erosion challenges on the collagen matrix.

SUMMARY

Objectives: To evaluate the effect of remineralizing agents on collagen matrix pattern, precipitate formation, and dentinal tubule obliteration in eroded cervical dentin.

Methods and Materials: One hundred bovine cervical dentin specimens were previously eroded

(0.6% hydrochloric acid, pH 2.3, 5 minutes) and then randomized into five groups (n=20): G1, control (without treatment); G2, Desensibilize Nano P (FGM); G3, MI Paste Plus (Recaldent); G4, Regenerate (NR-5); and G5, Desensibilize KF 2% (FGM). These treatments were applied in four sessions with 7-day intervals. During this period, the samples were subjected to an erosive

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challenge with orange juice (pH 3.8, 5 minutes). The specimens were analyzed by polarized light microscopy with picosirius red staining, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX).

Results: The G3 showed a higher concentration of type I collagen than G2 and G5 ($p < 0.05$). The G3 showed greater formation of surface precipitates than that of G1 and G5 ($p < 0.05$). In addition, G4 and G5 showed a greater number of open dentinal tubules than that of G3 ($p < 0.05$).

Conclusions: Calcium phosphate-based remineralizing agents have shown to be a promising alternative treatment for preventing deleterious effects on the eroded dentin collagen matrix. In addition, they promoted precipitate formation and dentinal tubule obliteration on the eroded dentin.

INTRODUCTION

A wide variety of products based on different technologies containing phosphate and calcium are commercially available, with the aim of providing greater resistance to demineralization of dental substrates or even recovering their mineralization. Among them, calcium- and sodium phosphate-based ones have been launched, focusing on erosion prevention and treatment. However, most of them are limited to their role on enamel and not on dentin.^{1,2} With regard to dentin treatment, greater complexity is involved, due to the participation and dynamics of organic content. In addition, the available evidence in this regard is limited.³ According to the best knowledge of the authors, to date, no evidence has been found regarding the effect of different remineralizing treatments on the profile of the eroded dentin collagen matrix.

One of the most frequent causes of dental tissue loss and exposure of cervical dentin is dental erosion.⁴ A previous study showed that gastroesophageal reflux (GR) of hydrochloric acid is directly related to dental erosion.⁵ At the same time, acidic foods and drinks have been increasingly included in current diets.⁶ Prevalence studies have shown that erosion caused by both intrinsic⁷ and extrinsic⁸ factors have high incidences. However, there are few materials on the market specifically indicated for the prevention and treatment of dental erosion. For this reason, alternative materials with remineralizing potential have been investigated.

Materials containing calcium phosphate nanoparticles in the form of nanohydroxyapatite (n-HAP) facilitate the deposition of precipitates on dentin and are recommended for dental remineralization in patients with dentin

hypersensitivity (DH).⁹ This action mechanism is related to the potential of stabilizing calcium and amorphous phosphate ions in dental structures.¹⁰ Another material studied is casein phosphopeptide-amorphous calcium phosphate associated with sodium fluoride (CPP-ACPF). The remineralizing mechanism of CPP-ACPF is related to the supply of calcium and phosphate ions on the tooth surface. In addition, it facilitates the stabilization of mineral precipitates that contain fluoroapatite.^{11,12} Its action mechanism is similar to n-HAP, and CPP-ACPF has also been recommended for the treatment of DH.¹¹⁻¹⁶

Calcium silicate and sodium phosphate (mono and trisodium phosphate) have been developed for the prevention and treatment of dental demineralisation.^{15,16} The probable action mechanism of this product is related to the deposition of calcium silicate particles and induction of hydroxyapatite formation on the dental substrate.¹⁷ This material can reduce dentin permeability and, therefore, it is a promising alternative for DH treatment.^{18,19} Potassium nitrate associated with sodium fluoride (NaF) is another material widely studied in the current literature, but its reported effects on DH have been controversial.^{20,21} Potassium ions (K^+) are the active component of this material and promote a reduction in the activity of dentinal sensory nerves due to the depolarizing activity of K^+ . On the other hand, NaF acts on tooth sensitivity by blocking exposed dentinal tubules and reducing the flow of fluid to the pulp.²²

Therefore, the aim of the present study was to evaluate the effect of remineralizing materials containing calcium phosphate nanoparticles (Desensibilize Nano P, FGM, Joinville, SC, Brazil), CPP-ACPF (MI Paste Plus Recaldent CPP-ACP, GC Company, Japan), calcium silicate associated with sodium phosphate (Regenerate NR-5, Unilever, Wirral, United Kingdom), and potassium nitrate associated with NaF (Desensibilize KF 2%, FGM) on the presence of collagen in eroded cervical dentin, the formation of precipitates, and obliteration of dentinal tubules. The null hypotheses tested were: H01—There are no differences in the presence of collagen in eroded cervical dentin under the different treatments tested; H02—There are no differences in the formation of precipitates and obliteration of dentinal tubules under the different treatments tested.

METHODS AND MATERIALS

Sample Preparation

One hundred bovine incisors with anatomically similar crowns and roots were stored in 0.1% thymol solution at 4°C until use. One hundred specimens (10 mm

long × 10 mm wide × 5 mm thick) were obtained from the cervical-third of the teeth using a cutting machine (Isomet 100, Buehler, Lake Bluff, IL) under constant refrigeration. The buccal surface of the fragments was polished on a polishing machine (DP-10; Panambra, Struers, Ballerup, DI) using a sequence of #600 and #1200 granulation sandpapers, for 20 seconds each, to expose the cervical dentin.

The dentin surface was previously eroded with 0.6% HCl and pH 2.3 (Arte & Ciência, Araraquara, São Paulo, Brazil) by immersion for 5 minutes. Then, the specimens were rinsed with distilled water and stored in artificial saliva (0.375 g/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.125 g/l $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.2 g/l KCl, 0.85 g/l NaCl, 2.5 g/l $\text{NaHPO}_4 \cdot 12\text{H}_2\text{O}$, 1 g/l sorbic acid, 5 g/l hydroxyethyl cellulose-sodium, and 43 g/l sorbitol solution) (Arte & Ciência) at 37°C until use.

Treatments and Erosive Challenges

The specimens were removed from the artificial saliva and slightly dried with soft paper. After that, the samples still wet received the application of the treatment materials. Table 1 shows the chemical composition of the remineralizing agents used in this study. Twenty specimens were used per treatment protocol (n=20), 10 specimens for polarized light microscopy, and 10 specimens for SEM/ energy-dispersive X-ray spectroscopy (EDX) analysis. The evaluated protocols were as follows:

- (G1): Specimens were kept in deionized water for 10 minutes.

- (G2): The product containing calcium phosphate nanoparticles (Desensibilize Nano P) was applied with a microbrush (KG Sorensen, São Paulo, SP, Brazil) and rubbed on the dentin surface with a rubber bowl (KG Sorensen) in low-speed rotation (500 rpm for 10 seconds). Then, the material was left in contact with the dentin for 5 minutes and was rinsed with 10 mL of deionized water, according to the manufacturer's recommendations.
- (G3): The material containing CPP-ACPF (MI Paste Plus Recaldent) was applied with a microbrush (KG Sorensen) and left in contact with the dentin surface for 3 minutes. The residues were then rinsed with 10 mL of deionized water.
- (G4): The material containing calcium silicate and sodium phosphate (Regenerate NR-5) was previously manipulated according to the manufacturer's guidelines, and subsequently applied with a microbrush (KG Sorensen) and left in contact with the dentin for 3 minutes. The residues were then rinsed with 10 mL of deionized water.
- (G5): The material containing potassium nitrate and 2% NaF (Desensibilize KF 2%) was applied to the dentin surface with a microbrush (KG Sorensen), without friction, and left in contact with the dentin for 10 minutes, according to the manufacturer's recommendations. The residues were then rinsed with 10 mL of deionized water.

Remineralizing treatments and erosion challenges were performed for 28 days. The specimens were

Table 1: Composition of Materials Used in the Study

Group	Composition	Manufacturer
G1: Control	Deionized water	—
G2: Desensibilize Nano P	Calcium phosphate (nano-HAP), potassium nitrate, and 9000 ppm NaF	FGM, Joinville, Brazil (Lot: 270417)
G3: MI Paste Plus Recaldent	CPP-ACPF, glycerol, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, sodium saccharin, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate, 900 ppm fluoride	GC, Tokyo, Japan (Lot: 1401221)
G4: Regenerate NR-5	Glycerin, calcium silicate, PEG-8, hydrated silica, trisodium phosphate, sodium phosphate, aqua, PEG-60, sodium lauryl sulfate, sodium monofluorophosphate, synthetic fluorophlogopite, sodium saccharin, polyacrylic acid, tin oxide, limonene, CI 77891, 1450 ppm fluoride	Unilever, Wirral, United Kingdom (Lot: 4175000)
G5: Desensibilize KF 2%	5% Potassium nitrate, 2% sodium fluoride (NaF), deionized water, glycerin, neutralizing and thickening agent	FGM, Joinville, Brazil (Lot: 131014)

Abbreviations: CPP-ACPF, casein phosphopeptide-amorphous calcium phosphate fluoride; NaF, sodium fluoride; PEG, polyethylene glycol.

submitted to four remineralizing treatment sessions, with 7-day intervals. Every day, the specimens were subjected to acid challenge by immersion in orange juice (Minute Mais, Coca Cola, SP, Brazil) at pH 3.80 for 5 minutes. After the erosive challenge, the samples were rinsed with 10 mL of deionized water and kept individually immersed in artificial saliva at 37°C. After each remineralizing treatment session, the specimens were subjected to the erosive challenge in the same way as the other days.

Polarized Light Microscopy Analysis

After undergoing the test protocols, the specimens were stored for 24 hours in artificial saliva. Then, they were placed in a 10% ethylenediaminetetraacetic acid (EDTA) solution (Merck, Darmstadt, Germany) at pH 7.0. This solution was changed twice a week for 90 days. After that, the specimens were rinsed again with distilled water, dehydrated in alcohol, diaphanized in xylol, and included in paraffin (Histosec, Merck, Darmstadt, Germany). The paraffin blocks were subjected to semiserial histological sectioning of 5- μ m thickness and submitted to the picosirius red staining technique.²³

After histological processing, each sample was analyzed using a microscope (Olympus BX51, Tokyo, Japan) equipped with filters to provide circularly polarized illumination. All image-acquisition parameters were standardized, and the intensity of illumination was calibrated by adjusting only the microscope's condenser opening. Digital images were obtained at 20 $\times$ magnification (DP-71, Olympus, Tokyo, Japan). The spectra of green, yellow, and red colors were defined following RGB values (red, green, blue) that were standardized for all images.

According to the orientation of the collagen fibers, the color of the spectrum can vary from greenish (type III collagen) to reddish (type I collagen).²⁴ To quantify only the greenish and reddish fibers, the images were analyzed using Affinity Photo Software (Serif Europe Ltd, Nottingham, United Kingdom), and the number of pixels for each color was calculated. In the sequence, the averages of the values obtained were calculated and expressed as percentages.

Scanning Electron Microscopy (SEM) Analysis

After completing the treatments described, the specimens were stored in artificial saliva for 24 hours. Then, they were dehydrated in a closed chamber with colloidal silica for 7 days. After assembling the specimens in metal stubs, they were metallized with colloidal gold (simple cycle: 120 seconds) under vacuum in a metallization chamber (MED 010,

Balzers Union, Balzers, Liechtenstein) and examined by SEM with JEOL 6060 (JEOL Ltd, Tokyo, Japan) operated at 20 kV, as described by Escalante-Otárola and others.²⁵

Representative images were obtained from the central region of each specimen. First, a 500 $\times$ magnification image was obtained to evaluate and quantify the precipitates formed on the dentin surface. Another image was obtained from the same location with 2000 $\times$ magnification, for counting open dentinal tubules. All images were obtained by a single operator.

Two properly calibrated independent examiners, using the weighted Kappa test ($\kappa = 0.82$), classified the presence of precipitates formed on the dentin surface according to the modified parameters described by Kuga and others.²⁶ (Scores from 0 to 4): Score 0, without precipitates; Score 1, any precipitates and all the tubules opened; Score 2, minimum amount of precipitates and > 50% of the dentin surface clean; Score 3, moderate amount of precipitates and < 50% of the dentin surface clean; Score 4, heavy precipitates with almost all tubules obstructed.

Energy-dispersive X-ray (EDX) Spectroscopy Analysis

Four specimens from each protocol were covered with carbon (BalTec SCD 004 Sputter Coater, Balzers) at 15 kV for 180 seconds. Then they were analysed by EDX (JEOL 6060; JEOL Co., Tokyo, Japan) to evaluate the main chemical components present on the dentin surface after performing the experimental protocols.

Statistical Analysis

Initially, all data obtained were subjected to the Shapiro–Wilk test to ascertain the data normality. Data on the dentin organic matrix were subjected to two-way ANOVA and Bonferroni tests. Data on the formation of precipitates and the number of open dentinal tubules (SEM) were subjected to Kruskal–Wallis and Dunn tests. All assessments were performed with a significance level of $\alpha = 0.05$.

RESULTS

Polarized Light Microscopy Analysis

G3 presented more type I (mature) collagen and less type III (immature) collagen than that in G2 and G5 ($p < 0.05$). In addition, G2, G3, and G5 did not show significant differences with the other groups ($p > 0.05$). Figure 1 shows the percentage of the presence of type I and type III collagen in the dentin substrate for each group. Figure 2 shows the incidence of type I (red

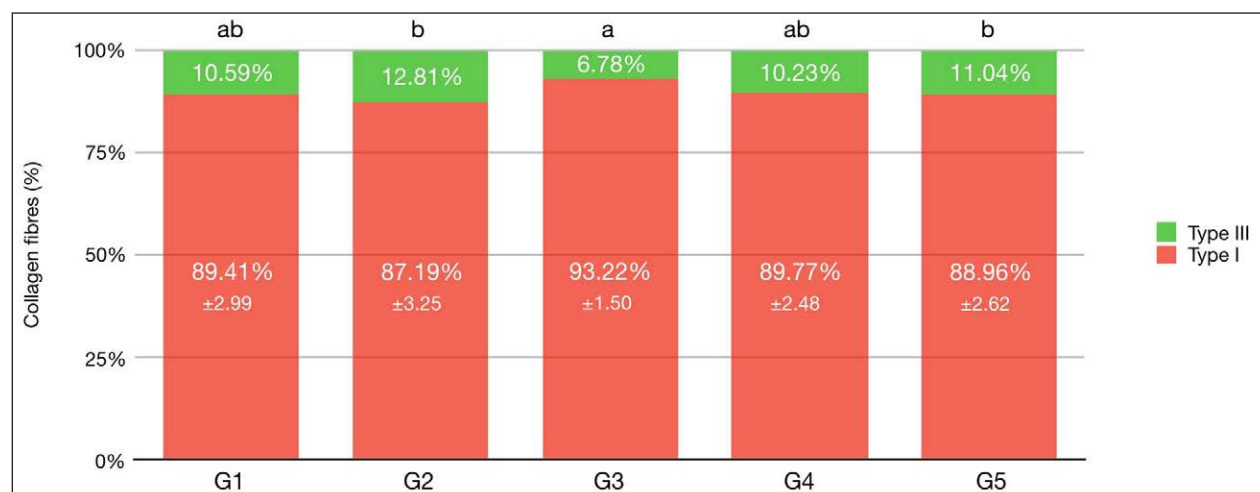


Figure 1. Results on the dentin organic matrix evaluation. a,b: Different letters indicate significant differences ($p < 0.05$). G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%.

shade) and type III (green shade) collagen in the dentin substrate after staining with picosirius red.

SEM Analysis

Table 2 shows the results of the formation of immediate precipitates in eroded cervical dentin and the occlusion pattern of dentinal tubules. Regarding the evaluation of dentin surface, G3 showed a higher incidence of precipitates on the dentin surface than that of G1 and G5 ($p < 0.05$). However, there was no difference in precipitate formation between G2, G3, and G4 ($p > 0.05$). On the other hand, there was no difference between G1 and G5 data compared to G2 and G4 ($p > 0.05$).

Regarding the number of open dentinal tubules, G3 presented the lowest incidence of open dentinal tubules when compared to that of G1, G4, and G5 ($p < 0.05$). However, there was no significant difference between G2 and G3 ($p > 0.05$). Figure 3 shows a representative image of the formation of precipitates on the surface (500 $\times$) and obliteration of dentinal tubules (2000 $\times$) for each evaluated group.

EDX Analysis

The EDX analysis showed that G3 presented higher concentrations of Ca/P (atomic ratio: Ca/P=1.93) when compared to the other groups. In addition, it demonstrated the presence of silicon (Si), magnesium (Mg), and sodium (Na). G1, G2, G4, and G5 groups showed the following concentration values between Ca/P atomic ratio: (G1), Ca/P=2.02; (G2), Ca/P=1.78; (G4), Ca/P=1.95; and (G5), Ca/P=1.89.

Figure 4 shows the relationship between Ca/P after the use of the tested materials. On the other hand, Figure 5 shows a representative image of the precipitate composition.

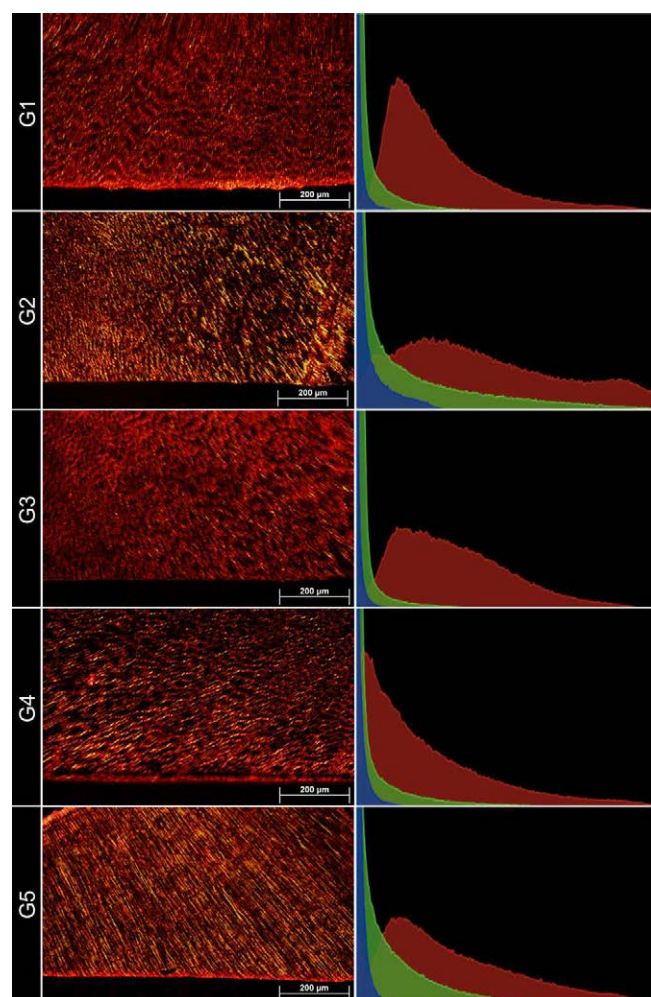


Figure 2. Representative image of the eroded cervical dentin organic matrix after treatments. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scale 200 mm.

Table 2: Quantitative Determination of Precipitates Formation (Average Scores) and Number of Open Dentinal Tubules ^a						
		G1	G2	G3	G4	G5
Precipitates over dentin (score)	Median	1 b	2 ab	3 a	2 ab	1 b
	Min/Max	1-2	1-3	2-3	1-3	1-2
	1Q/3Q	1-1	2-3	2-3	2-3	1-1
Open dentinal tubules	Mean	61.71 c	26.86 ab	18.57 a	43.14 bc	45.29 bc
	SD	6.90	8.57	4.76	9.12	9.55
Abbreviations: 1Q and 3Q, first and third quartile; Min, minimum value; Max, maximum value; SD, standard deviation; G1, Negative control; G2, Desensitize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensitize KF2%.						
^a Different lowercase letters on the same line indicate significant differences (p<0.05).						

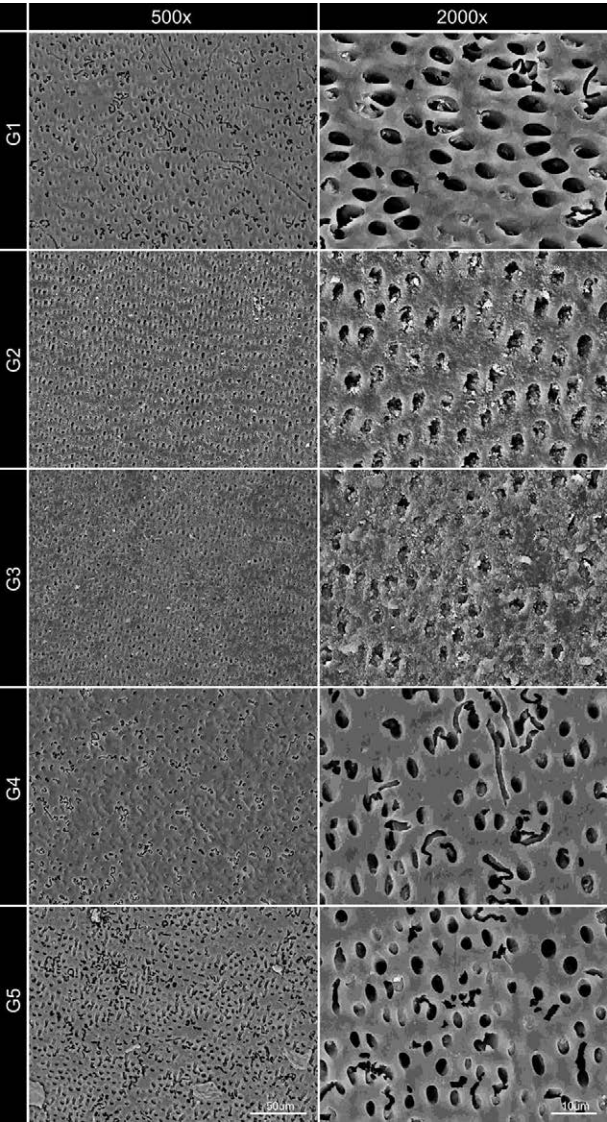


Figure 3. Representative image of the eroded cervical dentin surface treatments, at 500× and 2000×. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scales 50 mm and 10 mm.

DISCUSSION

Currently, the management of dental erosion is a challenge for the clinician, and several forms of treatment and prevention have been investigated.¹¹⁻¹⁵ The severity of erosive tooth loss depends on several parameters, such as quality and quantity of saliva, type of nutrition, and association with abrasion and mechanical friction.⁴ In the present study, remineralizing treatments were performed on previously eroded dentin. This methodology was carried out to simulate the clinical situation of patients with erosion, which is unlike many previous studies that carried out treatment experiments with healthy dentin.

The erosive demineralization of dentin results in the exposure of an outer layer of fully demineralized and often denatured organic matrix.²⁷ For this reason, the process of remineralization and biological reconstruction of this eroded substrate is crucial for its treatment.²⁸ The main organic component of dentin is type I collagen, which has a complex structure with four levels of hierarchical organization. One of the levels is in the molecular category formed by fibrils or collagen fibers.²⁹ These collagen fibrils are arranged both linearly and laterally in a hydrogel network under suitable physical–chemical conditions.³⁰ The distribution pattern of hydroxyapatite in type I collagen is distinguished in intrafibrillar and extrafibrillar mineralization. In the first case, hydroxyapatite is deposited within the gap area of the collagen fibrils, and, in the second case, it is deposited on the surface of the collagen fibrils.^{31,32}

Previous studies have already investigated the use of some materials with remineralizing potential on demineralized dentin; however, the investigations of these treatments on dentin collagen are scarce.^{33,34} Although a given material has good remineralizing properties on the dentin surface, it is crucial that it does not cause deleterious effects to the collagen matrix. In case of damage to the matrix, it may compromise the longevity of future restorations.^{25,35} In the present

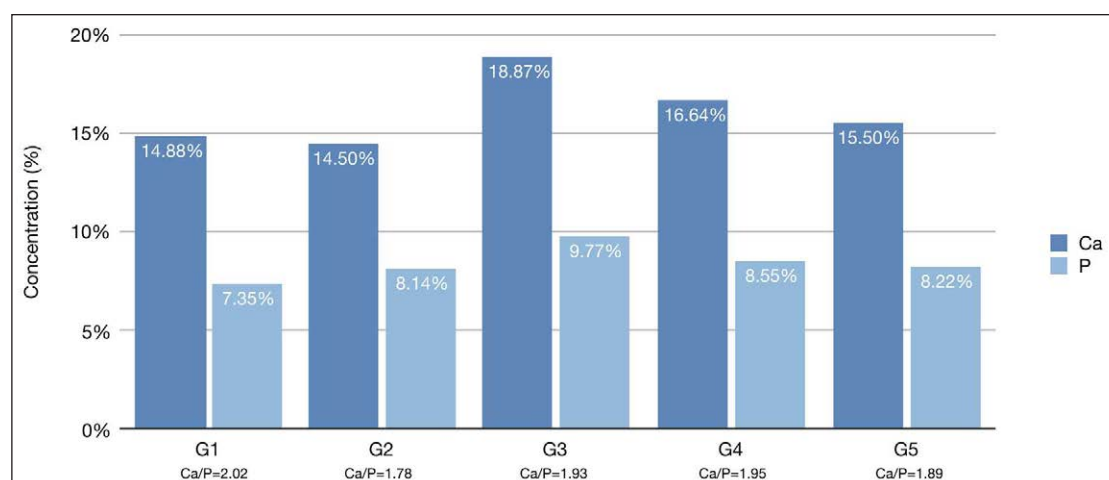


Figure 4. Images of X-ray energy dispersion [energy-dispersive X-ray spectroscopy (EDX)] analysis. Ca, calcium; Ca/P, atomic ratio; G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%; P, phosphorus.

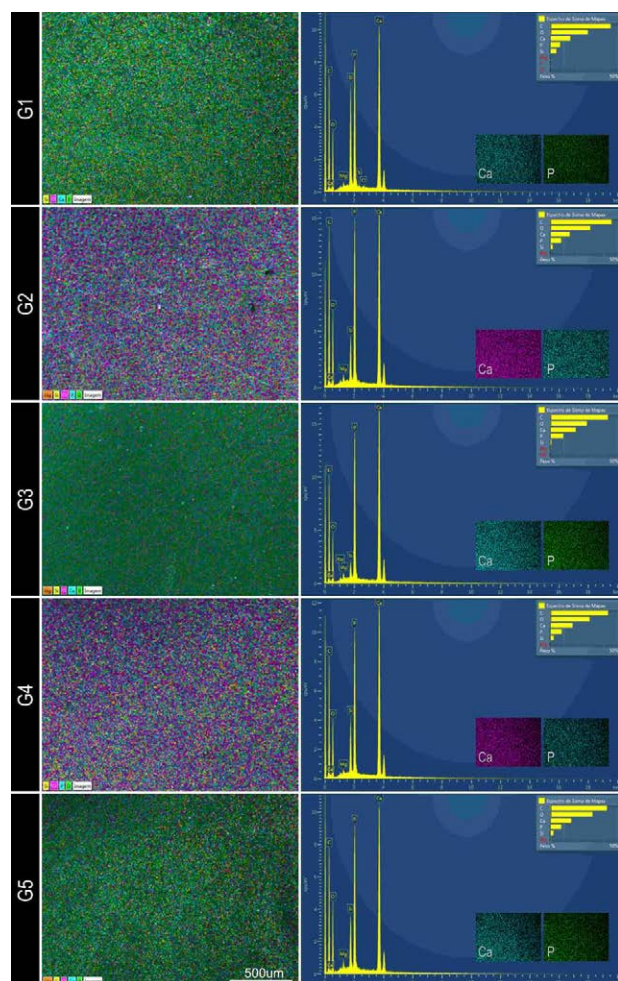


Figure 5. Image of X-ray energy dispersion [energy-dispersive X-ray spectroscopy (EDX)] analysis of the eroded cervical dentin surface after treatments. G1, Negative control; G2, Desensibilize Nano P; G3, MI Paste Plus Recaldent; G4, Regenerate NR-5; G5, Desensibilize KF2%. Scale 500 mm.

study, polarized light microscopy and staining with the picosirius red system was used to detect collagen, where red–yellow pigmentation and yellow–green pigmentation show type I and type III collagen, respectively. This method has been recommended by previous studies.^{23,24}

The results of the present investigation showed that the dentin substrate in G3 showed a higher incidence of type I collagen without abnormalities. However, in G2 and G5, there was a higher incidence of type III collagen, probably due to the presence of potassium ions, which can trigger changes in the organic matrix of the dental substrate.³⁶ Therefore, the null hypothesis H01 was rejected. A previous study conducted by Osorio and others³³ showed that the use of silver-loaded nanoparticles contributed to improve the interconnection, nature, and secondary structure of collagen in demineralized dentin. These results can be justified due to the use of polymeric nanoparticles, which are materials that sequester calcium and phosphate, facilitating the remineralization of dentin.^{37,38} In the present study, only the group treated with CPP-ACPF showed the type I collagen pattern without changes even in face of the erosive challenge. It is possible to hypothesize that the presence of calcium and phosphate ions in this material contributed to an effective remineralization and stabilization of type I collagen fibrils. However, further studies are necessary for a definitive conclusion on this issue.

In addition, G3 protocol demonstrated greater formation of precipitates on the dentinal surface and a lower incidence of open dentinal tubules than those of the G1 and G5. Therefore, the null hypothesis H02 was rejected. This can be explained by the mechanism of action of this material on eroded dentin. G3 contains in its chemical composition the CCP-ACPF and 900 ppm

of fluorine,^{39,40} and when applied to the dental substrate, it forms fluorapatite, which is a more stable and less soluble mineral than hydroxyapatite.^{41,42} In addition, the fluorapatite layer has acid-resistant properties,^{43,44} which could justify G3 results in this study.

EDX analysis showed a higher incidence of Ca and P on the dentin surface treated with CCP-ACPF, when compared to that of the other treatments. This finding shows that the presence of these ions is directly related to the presence of a layer of stable precipitates as well as resistance to the acid challenge in the dentin studied in G3. In addition, this material contains the milk protein casein, which could stabilize calcium phosphate in a solution and increase the level of calcium phosphate in enamel and dentin.⁴⁵ A previous study conducted by Alencar and others¹¹ evaluated the antierosive potential of CPP-ACPF on dentin, and concluded that this material is capable of obliterating dentinal tubules and promoting an increase in Ca and P ions on the dental surface. These results corroborate the findings of the present study.

G2 treated with n-HAP associated with 9000 ppm of NaF also showed promising results in the formation of superficial precipitates and the obliteration of dentinal tubules in this study. The nanoparticulate hydroxyapatite is morphologically similar to enamel apatite crystals and has high biocompatibility with dental tissues. Initially, n-HAP was studied as a biomimetic material for the reconstruction of lost enamel because of its high remineralizing potential.^{46,47} The phosphate and hydroxide anions released by n-HAP trap the hydrogen cations of the acid, and this reaction may be related to a decrease in the titratable acidity of saliva due to the increase in the concentration of n-HAP.⁴⁸ This could explain the antierosive effect of this material on dentin in the present study. However, a recent study showed that although n-HAP shows promising results in preventing dental erosion, it should not be used as the only treatment in the management of erosive tooth loss. It should be part of a preventive program associated with other strategies.¹² Another recent study showed that n-HAP applied only once did not show positive results in the obliteration of dentinal tubules.¹¹ It is possible that this material needs more applications to show an effective result.

The material used in G2 also contains 9000 ppm of NaF in its composition. It is possible that the remineralizing effect of NaF associated with n-HAP promoted a synergistic effect, potentiating the remineralizing effect of the material. NaF interacts with saliva and forms a layer of calcium fluoride (CaF_2) over the dentin. During an erosive challenge, this layer protects the dental structure from acid-induced degradation. Thus,

the presence of NaF in the oral cavity can prevent the acid from causing irreversible damage to dentin.^{49,50} On the other hand, this protective layer did not show a significant presence of Ca and P ions when compared to that of G3. It is possible that materials containing n-HAP and NaF are more susceptible to the erosive challenge than CPP-ACPF. This result corroborates with the findings of Alencar and others.¹¹

Although the compound with calcium silicate and sodium phosphate used in G4 induced the formation of precipitates on dentin, it demonstrated a high number of open dentinal tubules. This can be explained by the treatment strategy used in this study, as the manufacturer recommends home and daily use of the material.^{16,17} Therefore, it is possible that the precipitates formed were constituted by weak chemical bonds and present high sensitivity to the acid challenge. A study conducted by Poggio and others¹⁵ showed that regenerated toothpaste (NR-5) was able to prevent erosion in the face of an erosive challenge with soft drinks. However, that study carried out only one erosive challenge, while this present study subjected the samples to erosion for 28 days. The methodological differences in laboratory erosion studies hinder the interrelation between data presented in the literature.

G5 treated with 5% potassium nitrate associated with 2% NaF showed the worst antierosive results and was similar to the control group. Potassium nitrate acts specifically on the polarity of the nerve fiber stimulated during DH,²² and there is no evidence that it can form mineralized precipitates on the dental surface. Previous studies have shown that desensitizing toothpastes containing potassium nitrate have shown promising results in the obliteration of dentinal tubules.^{3,51} As already stated by some studies, they can promote effective obliteration when associated to other components.¹⁴ For this reason, studies that showed tubular occlusion after the use of materials containing Na^+ ions attributed this effect to the deposition of solid particles in the products and not to the influence of potassium.^{3,51} However, further studies must be carried out to clarify this issue.

The materials used in G2 and G3 proved to be remineralizing agents with potential antierosive effects. That is, they provide satisfactory precipitate formation in the intertubular dentin and occlusion of the dentinal tubules. Therefore, they can be an interesting alternative to assist in the prevention and treatment of dentin erosion. However, G2 showed a negative impact on the collagen matrix, unlike that of G3. Therefore, CPP-ACPF seems to be more indicated as an antierosive alternative, as it does not damage collagen. In addition, an evaluation of dentin permeability could complement

our results of dentin tubule obliteration, as it was done in preliminary studies on the use of desensitizing agents in eroded dentin.^{52,53} Future studies are necessary to corroborate and consolidate the results obtained in this study, as well as to deepen the knowledge about the impact of remineralizing agents on the dentin collagen matrix.

Saliva plays a significant role in the formation of the acquired film on the teeth. This semipermeable membrane makes it difficult for acid to contact the dental surface during an erosive challenge.⁵⁴ Therefore, one of the limitations of laboratory erosion studies would be the impact of this protective film on the observed results. To achieve this, all groups studied were subjected to the same conditions. In addition, it was observed that the control group showed significant superficial damage after the erosion challenge. This demonstrates that the saliva used in this study did not prevent erosive damage from occurring.

CONCLUSION

Calcium phosphate-based remineralizing agents showed to be a promising alternative treatment for preventing deleterious effects on the eroded dentin collagen matrix. In addition, they promoted precipitate formation and dentinal tubule obliteration on the eroded dentin.

Conflict of Interest

The authors declare that they have no conflict of interest. The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

Regulatory Statement

This study was conducted in accordance with all the provisions of the guidelines and policies of the Animal Ethics Committee of the Araraquara School of Dentistry, Paulista State University. The approval code issued for this study is No. 30/2018. PROSPERO acknowledgement of receipt is 158813.

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Three-dimensional Change of Elastomeric Impression Materials During the First 24 Hours: A Pilot Study

Howard Roberts

Clinical Relevance

Clinicians should be aware that volumetric polymerization shrinkage of elastomeric impression material continues for up to 24 hours after mixing. More accurate results may be obtained if impressions are poured as early as possible following the manufacturer's recommendations.

SUMMARY

Objectives: To evaluate the three-dimensional (3D) changes of three elastomeric impression materials using a novel measurement method for the first 24 hours after preparation.

Methods and Materials: Three impression materials consisting of a low-viscosity polyvinyl siloxane (PVS) (Aquasil LV, Dentsply Sirona, Charlotte, NC, USA) and two vinyl polyether silicone (VPES) materials consisting of a light body (EXA'lence LB, GC America, Alsip, IL, USA) and monophase (EXA'lence Monophase, GC America) materials were used in this study. All materials were prepared following manufacturer's recommendations with approximately 1-2 millimeters of material placed on the measurement pedestal of a calibrated noncontact,

video imaging based, volumetric change measuring device (AcuVol ver 2.5.9, Bisco, Schaumburg, IL, USA). Data collection was initiated immediately, with measurements made every 30 seconds for 24 hours. Each material was evaluated 10 times ($n=10$). Evaluated parameters included were 24-hour mean shrinkage, mean shrinkage at time of recommended first pour, mean shrinkage between recommended first pour and 24 hours, mean maximum shrinkage, and the time of maximum shrinkage. Mean data, both within and between each group, was evaluated using Kruskal–Wallis/Dunn's tests at a 95% level of confidence ($\alpha=0.05$).

Results: All three materials were found to have significant differences ($p<0.001$) in volumetric shrinkage over 24 hours. Aquasil LV and EXA'lence LB polymerization shrinkage rates were statistically similar all through the 24-hour evaluation ($p=0.92$). All three materials demonstrated similar ($p=0.19$) shrinkage between 10 and 15 minutes after preparation, while between 5 and 16 hours both EXA'lence Monophase and low-viscosity

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materials demonstrated similar polymerization shrinkage values ($p=0.22$). EXA'lence Monophase demonstrated significantly greater 24-hour mean shrinkage ($p<0.008$) as well as shrinkage between recommended first pour time and 24 hours ($p=0.003$) than Aquasil LV and EXA'lence LB. EXA'lence Monophase demonstrated significantly greater ($p=0.002$) shrinkage at the recommended time of first pour as compared to Aquasil LV and EXA'lence LB that displayed similar shrinkage ($p=0.89$). Furthermore, all materials demonstrated increasing polymerization shrinkage values that reached a maximum between 16 for Aquasil LV and 20 hours for EXA'lence LB, after which some relaxation behavior was observed. However, EXA'lence Monophase did not display any relaxation behavior over the 24-hour evaluation.

Conclusions: Under the conditions of this study, volumetric polymerization shrinkage was observed for one polyvinyl siloxane (PVS) and two vinyl polyether silicone (VPES) materials for up to 24 hours. All impression materials exhibited fast early volumetric shrinkage that continued past the manufacturer's recommended removal time. Dimensional change behavior was not uniform within or between groups; resultant volume change between the manufacturer recommended pouring time and 24 hours might represent up to from 20% to 30% of the total material shrinkage. It may be prudent to pour elastomeric impressions at the earliest time possible following the manufacturer's recommendations.

INTRODUCTION

Dental elastomeric impression materials should accurately reproduce intraoral features without distortion, as well as maintain a reasonable measure of stability after removal from the mouth.¹ Currently, polyvinyl siloxane (PVS) elastomeric impression materials are the predominant clinical choice for indirect restoration fabrication,²⁻⁵ with PVS material accuracy being well described,²⁻¹⁰ with some reports noting suitable linear dimensional stability for up to 14 days.^{3,4,7,11} PVS materials possess equivalent accuracy as compared to digital scanning technology^{7,9,12-19} but have been described as providing improved precision in situations involving multiple implant body, and dual-arch and full-arch impression requirements.²⁰⁻²⁴ PVS elastomeric impression materials are indeed subject to dimensional changes due to time-dependent polymerization shrinkage,^{3,25} constituent and product

material evaporation,^{11,26} as well as thermal differences between the intraoral and laboratory environment.²⁷⁻²⁹ PVS material stability may be further challenged by impression technique, material thickness control,^{28,30-32} as well as filler type and content.³³⁻³⁶

Current elastomeric impression material dental standards specify only linear dimensional stability requirements. American National Standard Institute/American Dental Association (ANSI/ADA) Specification Number 19 specifies that linear dimensional change can be no greater than 1.5%, which is usually assessed at 24 hours after preparation.³⁷ Accordingly, PVS linear dimensional stability has been reported.^{27,38-40} However, other researchers have studied three-dimensional (3D) stability using various methods to include calculated linear measurements, photometric topographical, and microtomographic (microCT) techniques.^{6,8,29,39,42} In addition, the time of stability assessments are various that have included: Immediately^{27,29,40,41-45}; 1 hour^{6,27,40,41}; 1.5 hours⁴⁴; 2 hours⁸; and 24 hours^{19,39,46} after preparation. To the author's best knowledge, no studies have reported real-time, 3D volumetric changes over the first 24 hours after preparation. The purpose of this study was to evaluate the real-time, 3D dimensional behavior of three impression materials utilizing a shrinkage evaluation method not previously used for elastomeric impression materials. The null hypothesis was that this methodology would find that there were no differences in the 3D dimensional stability between the impression materials being evaluated.

METHODS AND MATERIALS

The impression materials evaluated in this study were a low viscosity PVS material (Aquasil LV, Dentsply Sirona, Charlotte, NC, USA), a light body vinyl polyether silicone (VPES) material (EXA'lence LB, GC America Inc, Alsip, IL 60803 USA), as well as a monophase VPES material (EXA'lence Monophase, GC America). All the materials were prepared following manufacturer's instructions using supplied mixing tips. First, an initial amount of mixed impression material was extruded to ensure quality of mix. Next, 1-2 milliliters of material was placed on the polytetrafluoroethylene (PTFE) measurement pedestal in a calibrated (eg, gray scale and pedestal position) noncontact, video imaging based, volumetric change measuring device (AcuVol ver 2.5.9, Bisco, Schaumburg, IL, USA). Figure 1 depicts a representative image of an impression material placed on the measurement pedestal, while Figure 2 displays the measurement screen as seen on the computer monitor. The yellow line at the base of the impression material represents the pedestal outline,

which was predetermined before material placement, while the green line outlines the impression material to be analyzed. Data collection was initiated immediately after placement with volume measurements made every 30 seconds for 24 hours. Each material was evaluated 10 times ($n=10$). Evaluated parameters included 24-hour mean shrinkage, mean shrinkage at the manufacturer's recommended pour time, mean shrinkage between the manufacturer's recommended pour time and 24 hours, the mean maximum shrinkage, and the time of maximum shrinkage. Furthermore, volumetric shrinkage was analyzed every minute during the first 5 minutes, followed by evaluation every 5 minutes up to 30 minutes. Mean data was found to contain abnormalities in both data distribution as well as variance homogeneity by the Shapiro–Wilk and Bartlett Test, respectively. Therefore, data was analyzed by the Kruskal–Wallis test with Dunn's post hoc analysis. All analysis was performed at a 95% level of confidence ($\alpha=0.05$)

RESULTS

Comparative summary results are shown in Table 1, with mean results initially displayed every minute for the first 5 minutes. Then results are listed at 5-minute intervals until 30 minutes after preparation, followed by mean results being displayed every 30 minutes until up to 6 hours after preparation. Thereafter, hourly mean results are posted up to 24 hours. All three impression materials demonstrated significant



Figure 1. Impression material placed on measurement pedestal. Impression material (green) contoured to fit on measurement pedestal (white).

differences in volumetric shrinkage over the 24-hour evaluation period. All three materials were hallmarked with similarity in shrinkage values; the high observed covariance undoubtedly contributed to this outcome. However, numerical trends can be observed when shrinkage change slowly subsides. This can be conjectured starting at approximately 8 hours for EXA'lence LB, 11 hours for Aquasil LV, but the EXA'lence Monophase trend requiring up to approximately 19 hours after preparation.

Comparison between materials found that Aquasil LV and EXA'lence LB polymerization shrinkage were statistically similar ($p=0.92$) all through the 24-hour evaluation. All three materials demonstrated similar shrinkage ($p=0.19$) between 10 and 15 minutes after preparation, while both EXA'lence viscosities had similar ($p=0.22$) polymerization shrinkage values between 5 and 16 hours. The mean real-time shrinkage data over the 24-hour evaluation is graphically displayed in Figure 3.

The volumetric polymerization shrinkage behavior during the first 30 minutes are detailed in Figure 4.

EXA'lence monophase demonstrated the highest initial volumetric polymerization shrinkage that transformed into a slower rate, starting approximately 8 minutes after material preparation that was observed to change to a slower rate at 12 minutes. Aquasil LV displayed a slower polymerization shrinkage change but somewhat mimicked, albeit on a lesser scale, the rate change behavior of EXA'lence Monophase. EXA'lence LB demonstrated the slowest initial polymerization shrinkage; however, this rate did not display as a dramatic rate transformation like the others but continued and surpassed Aquasil LV at approximately 15 minutes after preparation.

Afterwards, all three materials continued to demonstrate volumetric polymerization shrinkage, with maximum shrinkage occurring at approximately 16 hours after preparation with Aquasil LV exhibiting

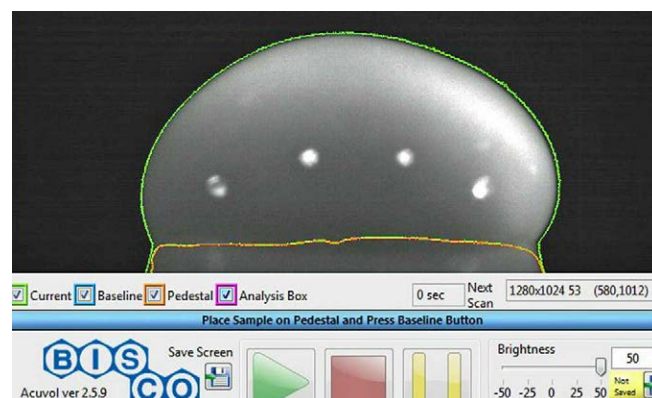


Figure 2. AcuVol measurement screen.

Table 1: Mean Shrinkage (%) with Time^a

Time (hours)	Aquasil LV	EXA'lence LB	EXA'lence Monophase
0	0.00 A	0.00 A	0.00 A
0.01 (1m)	0.08 (0.1) Aa	0.06 (0.01) ABa	0.24 (0.1) ABb
0.03 (2m)	0.16 (0.1) ABa	0.16 (0.1) ABCab	0.38 (0.2) ABCb
0.05 (3m)	0.28 (0.1) ABa	0.21 (0.1) ABCDa	0.55 (0.1) ABCDb
0.06 (4m)	0.38 (0.1) ABCDa	0.21 (0.1) ABCDEa	0.66 (0.1) ABCDEb
0.08 (5m)	0.44 (0.1) ABCDEa	0.33 (0.1) ABCDEa	0.79 (0.2) ABCDEb
0.16 (10m)	0.56 (0.1) ABCDEFa	0.51 (0.2) ABCDEa	1.08 (0.4) ABCDEa
0.25 (15m)	0.57 (0.2) ABCDEFa	0.59 (0.2) BCDEa	1.16 (0.4) BCDEa
0.5	0.62 (0.1) BCDEFa	0.73 (0.2) BCDEa	1.13 (0.4) BCDEb
1	0.65 (0.2) CDEFa	0.75 (0.2) BCDEa	1.09 (0.3) BCDEb
1.5	0.67 (0.2) DEFa	0.78 (0.2) BCDEa	1.13 (0.4) BCDEb
2	0.70 (0.2) DEFa	0.78 (0.3) BCDEa	1.08 (0.3) BCDEb
2.5	0.70 (0.2) DEFa	0.80 (0.3) BCDEa	1.12 (0.4) BCDEb
3	0.73 (0.2) DEFa	0.79 (0.3) CDEa	1.15 (0.3) BCDEb
3.5	0.75 (0.2) DEFa	0.82 (0.3) CDEa	1.15 (0.3) CDEb
4	0.75 (0.2) DEFa	0.85 (0.3) CDEa	1.20 (0.4) CDEb
4.5	0.74 (0.2) DEFa	0.86 (0.3) CDEa	1.19 (0.3) CDEb
5	0.77 (0.2) EFa	0.88 (0.3) DEFGab	1.17 (0.4) CDEb
5.5	0.77 (0.2) EFa	0.91 (0.3) DEab	1.27 (0.4) DEb
6	0.75 (0.2) EFa	0.89 (0.3) DEab	1.28 (0.5) DEb
7	0.78 (0.2) Fa	0.94 (0.3) DEa	1.35 (0.5) DEb
8	0.77 (0.2) Fa	0.99 (0.3) DEa	1.35 (0.56) DEab
9	0.78 (0.2) Fa	1.0 (0.3) Eab	1.42 (0.6) DEb
10	0.79 (0.2) Fa	1.0 (0.3) Eab	1.41 (0.5) Eb
11	0.83 (0.2) Fa	1.05 (0.3) Eab	1.40 (0.4) Eb
12	0.85 (0.2) Fa	1.01 (0.3) Eab	1.42 (0.4) Eb
13	0.84 (0.2) Fa	1.01 (0.3) Eab	1.40 (0.4) Eb
14	0.85 (0.2) Fa	1.01 (0.2) Eab	1.40 (0.4) Eb
15	0.85 (0.2) Fa	1.0 (0.3) Eab	1.43 (0.5) Eb
16	0.83 (0.2) Fa	1.0 (0.3) Eab	1.46 (0.5) Eb
17	0.82 (0.2) Fa	1.0 (0.2) Ea	1.51 (0.5) Eb
18	0.87 (0.1) Fa	1.0 (0.3) Ea	1.55 (0.5) Eb
19	0.85 (0.2) Fa	1.01 (0.3) Ea	1.64 (0.6) Eb
20	0.84 (0.2) Fa	0.96 (0.3) Ea	1.65 (0.6) Eb
21	0.84 (0.2) Fa	0.98 (0.3) Ea	1.60 (0.5) Eb
22	0.83 (0.2) Fa	0.98 (0.3) Ea	1.67 (0.6) Eb
23	0.82 (0.2) Fa	0.95 (0.3) Ea	1.65 (0.6) Eb
24	0.78 (0.1) Fa	0.93 (0.3) Ea	1.66 (0.6) Eb

^aUppercase letters identify similar groups per column. Lowercase letters identify similar groups per row.

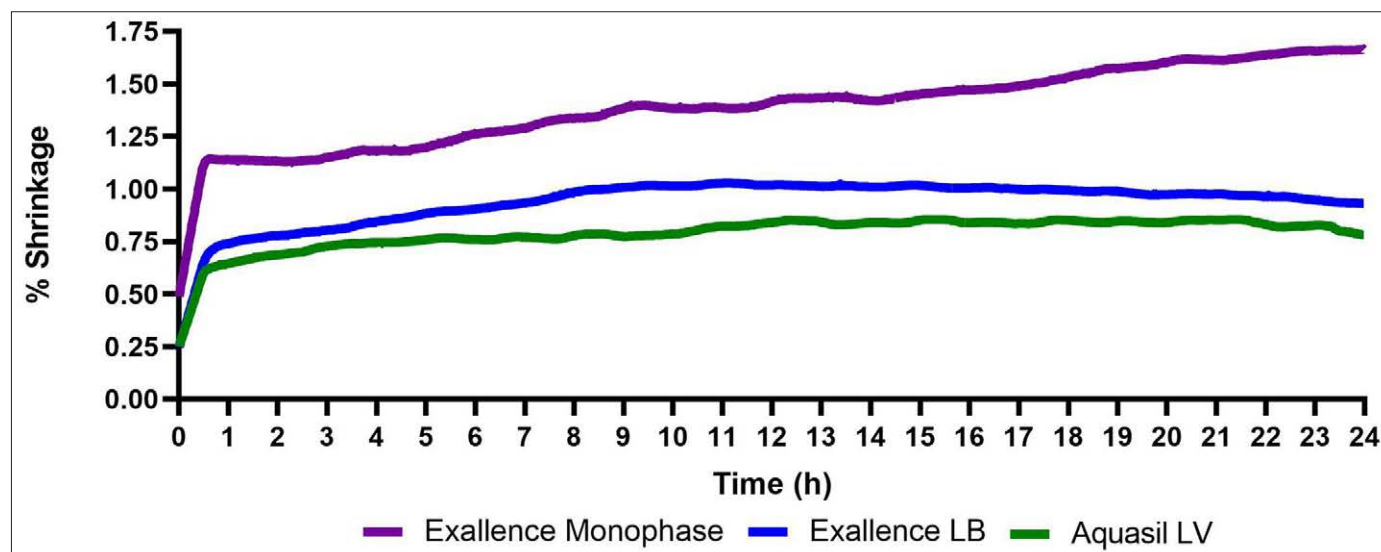


Figure 3. Mean real time data graphs. $n = 10$; 0th Order Savitzky-Golay smoothing.

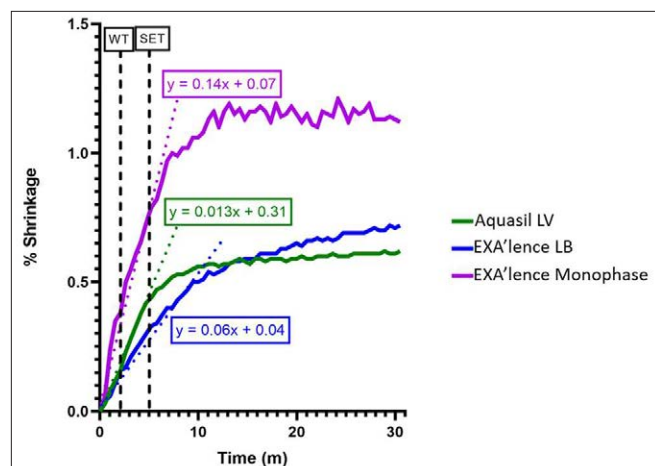


Figure 4. Early polymerization shrinkage data (%). $n = 10$; raw data not smoothed. Polymerization shrinkage slope (color-coded dotted line, formula); WT = Vertical dashed line identifies end of working time for all materials; SET = Vertical dashed line identifies manufacturer's recommended removal from mouth time.

maximum shrinkage at 16 hours after preparation, while EXA'lence LB followed suit at 20 hours. EXA'lence Monophase displayed variable behavior but did not demonstrate any relaxation over 24 hours.

The mean 24-hour polymerization shrinkage, mean polymerization shrinkage at recommended pour time, mean shrinkage between recommended pour time and 24 hours, maximum shrinkage, and time of maximum shrinkage is shown in Table 2. EXA'lence Monophase demonstrated a significantly greater 24-hour mean shrinkage ($p < 0.008$) as well as shrinkage between the recommended pour time and 24 hours ($p = 0.003$) than Aquasil LV and EXA'lence LB. EXA'lence Monophase

demonstrated significantly greater ($p = 0.002$) shrinkage at recommended time of first pour as compared to Aquasil LV and EXA'lence LB, which displayed similar volumetric shrinkage ($p = 0.89$). Furthermore, EXA'lence Monophase also displayed a significantly greater mean maximum shrinkage ($p < 0.002$) than the similar Aquasil LV and EXA'lence LB ($p = 0.67$), but there was no significant difference in the time that the maximum shrinkage occurred ($p = 0.89$).

DISCUSSION

PVS impression materials have a documented market tenure of linear dimensional stability.^{2,3,7-9,12-17} PVS base materials usually consist of a polymethyl hydrogen siloxane copolymer and an accelerator containing polydimethylsiloxane and a chloroplatinic acid metal complex catalyst.² Notably hydrophobic due to the aliphatic hydrocarbon chains surrounding the siloxane bond, PVS materials were modified with intrinsic surfactants,⁴⁷ ostensibly to afford better stone-pouring behavior and gypsum compatibility.⁴⁸ VPES materials were introduced as an impression material that would be intrinsically hydrophilic without added surfactants that would also provide the clinical handling of PVS materials.¹¹ The manufacturer reports that EXA'lence materials contain a 5%-20% polyether component, which is presumably responsible for enhancing the hydrophilicity of the impression material.¹¹ The PVS component in VPES materials is reported to contain both polyvinyl dimethyl and methyl hydrogen components,¹¹ and VPES accuracy has been reported in several studies.^{11,42,44,46,49,50} It should be noted that both PVS and VPES base and accelerator pastes also contain fillers that

Table 2: Mean Results^a

	Aquasil LV	EXA'lence LB	EXA'lence Monophase
Mean 24-hour Shrinkage (%)	0.76 (0.14) a	0.89 (0.2) a	1.43 (0.5) b
Mean Shrinkage at Recommended Pour Time (%)	0.62 (0.2) a	0.75 (.2) a	1.14 (0.4) b
Mean Shrinkage Between Recommended Pour Time and 24 Hours (%)	0.16 (0.1) a	0.18 (0.2) a	0.52 (0.3) b
Mean Maximum Shrinkage (%)	1.04 (0.2) a	1.19 (0.3) a	1.93 (0.7) b
Mean Time of Maximum Shrinkage (Hours)	18.1 (4.5) a	16.4 (5.4) a	— ^b

^aLowercase letters denote similar groups per row (Kruskal-Wallis/Dunn's, $n=10$, $p=0.05$).

^bNot determined.

are added to modify handling properties, mechanical properties, and dimensional stability.⁵¹ Depending on content and morphology, different filler materials can interface and physically interact with the impression material polymer chains, and improve mechanical properties and stability.^{35,36,51,52} Furthermore, some filler materials have been reported to be surface treated to improve interactions with the polymer chains.³⁶ The Safety Data Sheet (SDS) does identify that Aquasil LV may contain up to 60%-70% silicon dioxide fillers,⁵³ while EXA'lence materials are said to contain up to 65% silicone dioxide fillers.¹¹ The precise etiology for the noted volumetric polymerization shrinkage difference between the two VPES materials is not precisely known, but speculatively manufacturers can manipulate both polymer molecular weight and the filler constituent to achieve the desired material mechanical properties.

Aquasil LV has a stated working time of 1 minute and 10 seconds, while both VPES materials are described to have 2 minutes of working time.^{54,55} All three products are said to be ready for removal from the mouth at 5 minutes after material preparation.^{54,55} As evidenced in Figure 4, all materials still experienced a considerable volumetric polymerization shrinkage at the manufacturer's recommended mouth removal time. Concern may exist with the introduction of removal distortion forces interfering with polymer chain polymerization and crosslinking. While the added effects of distortion are not fully appreciated, infrared Fourier transform spectroscopy (FTIR) evidence from Derchi and others⁵⁶ suggests that the majority of Aquasil polymer crosslinking actions has been accomplished by 300 seconds after material preparation. However, no similar studies with the VPES materials can be found in the literature.

For model fabrication, Aquasil LV is said to be able to be poured with gypsum stone 30 minutes after disinfection, while it is recommended to wait up to 60 minutes for an epoxy material.⁵⁴ The manufacturer recommends that both EXA'lence materials be poured 60 minutes

after mouth removal.⁵⁵ At these recommended gypsum pouring times, all the materials are beyond the observed initial faster changes of polymerization shrinkage. However, between the manufacturer's recommended pouring time and 24 hours later, both Aquasil LV and EXA'lence LB exhibited an additional 0.16% and 0.18% volumetric shrinkage, respectively, which represents approximately 20% of the total 24-hour shrinkage for both materials. Over the same time, EXA'lence Monophase displays an additional 0.57% polymerization shrinkage, which represents over 34% of the material's total shrinkage. While all the materials were noted to exhibit continued volumetric polymerization shrinkage, demonstrated points of maximum shrinkage were observed at approximately 16 hours for Aquasil LV and 20 hours for EXA'lence LB. This volumetric shrinkage recovery occurred perhaps related to relaxation of internal stresses in the polymer network.¹ However, any reduction did not compensate to values observed at the recommended pour time. Any shrinkage recovery with EXA'lence Monophase was not observed during the 24-hour evaluation time.

The null hypothesis was rejected, as significant difference in volumetric polymerization shrinkage behavior was observed. Direct comparison with other studies describing 3D impression material shrinkage analysis is difficult due to the introduction of the technology used in this study. However, using the linear results of diameter and height change reported by Gomez-Polo and others,⁴⁰ a calculated 3D shrinkage change of a low-viscosity PVS material was approximately 0.22% between 1 and 24 hours. While this is indeed similar to that observed with Aquasil LV and EXA'lence LB in this study, the author assumes this similarity with caution, as different materials and testing methodologies were used.

Under the conditions of this study, the analysis method identified that a mean numerical 24-hour linear dimensional shrinkage assessment may not accurately

reflect the dimensional change dynamics demonstrated by elastomeric impression materials. Vasiliu and others⁵⁷ noted that elastic impression material contraction was not uniform during the first 24 hours after removal from the oral cavity. Accordingly, Chandran and others³⁹ reported both 2D and 3D impression material shrinkage results, and found that while the materials easily met 2D linear requirements, 3D analysis identified volumetric distortion in some situations as great as 100 μm . Furthermore, Rodriguez and Bartlett⁵⁸ evaluated impression material stability, and discovered differences between linear and 3D methods, concluding that the 3D shape of a model influences impression material shrinkage over time. Levartovsky and others⁵⁹ compared linear methods and a 3D tooth-simulating model, and found that information derived from the 3D model analysis suggested that impressions derived from a one-step technique should be poured within 2 hours for the best accuracy, while materials and techniques met all linear dimensional change requirements.⁵⁹ This was reinforced by Garg and others,⁶⁰ who noted that single-step monophasic viscosity impression material impressions demonstrated more distortion and suggested that pouring of the impression should not be delayed. Nassar and others¹¹ noted that VPES materials provided good dimensional stability over a period of time. However, 3D measurement of produced die materials suggested that the best accuracy was exhibited at the recommended immediate pour time.¹¹ The present study's data suggests that gypsum cast accuracy may depend on the chosen time that the impression is poured after mouth removal, as the volumetric polymerization shrinkage continued to progress with time. It may be considered prudent to pour elastomeric impression materials at the earliest possible time recommended by the manufacturer.

Under the conditions of this study, the data suggests that by delaying pouring of an impression until 24 hours after mouth removal, the continued volumetric shrinkage might afford a resultant gypsum preparation exhibiting up to 0.2% and 0.3% volume difference. Pereira and others¹² reported that a polyether material provided a gypsum die that was larger than the master die. The authors reasoned that a slightly larger indirect restoration could be advantageous as to possibly providing more space for cement.¹² However, this finding was contrasted by Emir and others,⁸ who reported that volumetric changes resulted in smaller definitive casts as compared to a master model. While differences in restoration size could hopefully be reconciled with adjustment of cast metal and/or metal-ceramic crowns, evidence is emerging that identifies the importance of an accurately designed

cement space with all ceramic crowns. Reports suggest possible etiology with monolithic ceramic crown clinical failures that originate from adhesive is due to radial forces that originate from adhesive cement polymerization stresses due to uneven and/or thicker adhesive resin-cement thickness between the tooth preparation and the intaglio ceramic crown surface.⁶¹⁻⁶³

Limitations to this pilot study include the high data covariance that will be addressed in follow-up studies by using this study's data for a power analysis. Improved data acquisition with hopeful covariance reduction may also be addressed by exploring methods to reduce the impression material's surface reflectivity. Reduction of glare from the material surfaces may reduce the varied readings by the device's sensitive video camera. Furthermore, future evaluations should use material from multiple impression material cartridges to improve the number of independent samples. Linear polymerization shrinkage was not assessed for comparison and will be included in future evaluations as well as a possible control material. While this method's resultant data cannot be considered absolute, methods of calibration for this technology will be refined. Furthermore, these preliminary results may only apply to the materials tested, and should not be assumed to represent all PVS and VPES materials.

CONCLUSION

Under the conditions of this study, volumetric polymerization shrinkage was observed for one PVS and two VPES materials for up to 24 hours. All impression materials exhibited fast early volumetric shrinkage that continued past the manufacturer's recommended removal time. Dimensional change behavior was not uniform within or between groups' resultant volume change between the manufacturer's recommended pouring time, and 24 hours might represent up to 20%-30% of the total material shrinkage. Comparative literature identifies that linear polymerization shrinkage assessment methods may not accurately reflect impression material 3D dynamic shrinkage behavior. Evidence is presented that indicates it may be prudent to pour elastomeric impressions at the earliest time possible, following the manufacturer's recommendations. The clinical implications of these findings are not currently appreciated, and continued work in this area to improve the experimental technique and evaluate more impression materials is recommended.

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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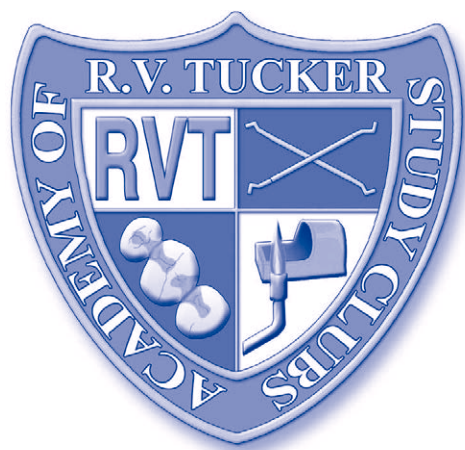
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